SUPPRESSION OF *MELOIDOGYNE* SPP BY *PASTEURIA PENETRANS*

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Before you can reach to the top of a tree and understand the buds and flowers
you will have to go deep to the roots, because the secret lies there
And the deeper the roots go the higher the tree goes

Nietzsche

To a man who taught me many of my skills, my father, Armin Willi Paul Weibelzahl

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Suppression of plant-parasitic nematodes with microbial agents is an alternative or supplemental management tactic that is receiving increased interest among nematologists. One nematode antagonist, the endospore forming bacterium *Pasteuria penetrans*, has shown great potential in suppressing field populations of several plant parasitic nematodes throughout the world. This obligate parasite of mainly root-knot nematodes, *Meloidogyne* spp., was studied for its potential to suppress *M. arenaria* on peanut, and *M. incognita* and *M. javanica* on tobacco. The objectives were to monitor the population densities of the root knot nematode and *P. penetrans* in peanut microplots and in a naturally infested tobacco field; to develop a suppressive-soil test that allows the determination of the role of *P. penetrans* in nematode-suppressive soils; and to evaluate the effect of cultural practices, such as crop rotation, autumn cover crops, resistance and fertilizer regimes on the abundance of *P. penetrans*. Within 4 years after inoculation or contamination by *P. penetrans*, *M. arenaria* was nearly eliminated from microplot soil, and peanut yields were similar to that of the nematode free control. The *P. penetrans* infested soil remained suppressive to *M. arenaria* for 3 years. Similar results were observed in a tobacco field.

After 4 years of tobacco monoculture, a mixed population of *M incognita M javanica* and *M arenaria* was suppressed to non-damaging levels Two *P penetrans* isolates were more pathogenic to *M incognita* than to *M javanica* Inorganic nitrogen fertilizer rates and host resistance to *M incognita* had inconsistent effects on the endospore build up which was favored by cultivation of susceptible cover crops A microwave radiation treatment of 3 minutes/kg of soil containing 6% to 7% water reduced the fungal population in soil samples without impairing attachment of *P penetrans* endospores to nematode juveniles This treatment allowed the separation of nematode suppression by fungi from that caused by *P penetrans* Nematode suppressiveness in microplot and field soil was preserved after microwave and air-drying treatments, but not after soil was autoclaved. The bacterial parasite *P penetrans* was identified as the main contributor to the suppressiveness of the microplot and the field soil

CHAPTER 1
INTRODUCTION

Biological Control of Nematodes

Introduction

Nematologists are continuously unveiling the biological control potential of *Pasteuria penetrans* (Thorne 1940) Sayre and Starr 1985, a mycelial endospore forming bacterial parasite of mainly root-knot nematodes. The ecological history of *P. penetrans* in association with *Meloidogyne* spp., as well as knowledge about its role in nematode management strategies, is essential for improving the use of the organism as an effective biological control agent. This information also is important for recognition and effective exploitation of soils naturally infested with *P. penetrans*.

In 1991, soils suppressive to the peanut root knot nematode *M. arenaria* (Neal) Chitwood race 1, were produced in microplots at the University of Florida Agronomy Farm, Green Acres, Alachua County, Florida. Ninety microplots (76 cm in diameter) were established with 30 inoculated centrally with relatively low numbers of *P. penetrans* and *Meloidogyne arenaria*, 30 inoculated with *M. arenaria* alone, and 30 left uninoculated as control plots (Oostenderp et al., 1991a). The microplots were planted to peanut in the spring of 1987. Within 3 years, yields of plots infected with *P. penetrans* and *M. arenaria* increased to a level comparable to the untreated control and the damage from root knot nematodes decreased, suggesting that the soil became suppressive to the nematode. A

minimum of 3 years of intensive cropping was required for *P penetrans* to amplify to suppressive levels During the same period, a tobacco field that was naturally infested with *Meloidogyne* spp was discovered to contain a soil suppressive to the nematode This site was located about 300 meters from the microplot site. The bacterial parasite *P penetrans* was reported to cause the nematode suppression (Chen et al , 1994)

The goal of this project was to monitor the dynamics of the bacterium in the two sites and further establish the relationship of *P penetrans* to suppressive soil

Historical Background

Biological control of nematodes has been of great interest to nematologists for almost as long as plant-parasitic nematodes have been known to damage crops (Stirling 1991) Cobb (1920) considered using parasites and predators of nematodes as biological control agents when he suggested transferring predacious nematodes to sugarbeet fields in order to control populations of *Heterodera schachtii* Schmidt. Thorne (1927) however, questioned the economic benefits of predacious nematodes, and further studies on their potential for biological control of plant parasitic nematodes came to an end. Linford s (1937, 1939) early efforts to use predacious fungi to control plant parasitic nematodes stimulated interest in the nematode-trapping fungi in France the United States, England, and the former USSR (Stirling, 1991) Due to the lack of success of these early experiments the interest in biological control declined It was not until the late-1970s that interest in biological control revived when the potential health and environmental problems associated with the use of some nematicides surfaced (Kerry 1990, 1993, Stirling 1991 Thomason, 1987) The suspension of two of the most reliable soil fumigants DBCP (1 2 dibromo-3-chloropropane) and EDB (ethylene dibromide), and the lack of promising new

candidate nematicides, led nematologists to re-evaluate their goals in terms of nematode management research. This fact, plus the classical studies that demonstrated fungal antagonists suppressed populations of *Heterodera avenae* Woll. in Europe (Kerry et al. 1980, 1982) and other studies showing that fungal antagonists also suppressed *Meloidogyne* spp. (Stirling and Mankau, 1978, Stirling et al., 1979), rekindled interest in biological control. In recent years, some attempts have been made to market various fungal agents for nematode control (Al Hazmi et al., 1993, Timm, 1987), but the products generally have not been accepted.

Unfortunately, there are still no widely used examples of the contrived use of biological antagonists to control plant parasitic nematodes. Although there are many likely candidates (Dickson et al., 1994, Jansson, 1988, Kerry, 1987, 1988, Mankau, 1975b, 1980b, Sayre et al., 1988, Stirling, 1988, 1991, Tribe, 1980), the resources devoted to research on biological control of nematodes have been relatively limited (Stirling, 1991). There is optimism, however, that biological control will play an increasingly important role in future nematode management programs.

The search for nematode antagonists has generally centered on predacious and parasitic fungi and bacterial parasites. One such bacterium, *P. penetrans*, was identified and considered to have great potential for biological control of root-knot nematodes (Dickson et al., 1994). A *Pasteuria* sp. was first associated with parasites of plant parasitic nematodes when Thorne (1940) reported it as *Duboscqia penetrans* parasitizing *Pratylenchus penetrans*. Since the first reports that *Pasteuria penetrans* parasitized root-knot nematodes (Mankau, 1980a, 1980b, Stirling and White, 1982, Stirling, 1984), the organism became the subject of an increasing number of research projects (Stirling, 1991). *Meloidogyne* spp. were reported to be suppressed successfully in numerous pot, microplot, and field experiments (Brown et al., 1985, Channer and Gowen, 1988, Chen et al., 1996b,

1987c Dandi, 1990 Dube and Smart, 1987, Jaya Raj and Mani 1988 Maheswari and Mani, 1988, Maheswari et al., 1987 1988, Oosterdorp et al., 1991a Stirling 1984 Tzortzakakis 1994a Vargas et al 1992 Weibelzahl Fulton et al 1996) However limitations in mass production methods of *P penetrans* have prevented its commercialization

Taxonomic Status of the Genus *Pasteuria*

The genus *Pasteuria* comprises a group of Gram positive mycelial and endospore forming bacteria that parasitize bodies of invertebrates including nematodes (Williams et al 1989a) The initial studies of the genus *Pasteuria* date back to 1888 when Metchnikoff described an internal parasite of two *Daphnia* spp (water fleas) as *Pasteuria ramosa* (Metchnikoff 1888) Partial credit for the discovery of the bacterium also should be given to Cohn (1906) who noted *Pasteuria* sp. as an internal parasite of nematodes sketched it, and described it to be "perhaps monads" The taxonomy of this nematode parasite however has been subject to continuous confusion ever since Thorne (1940) described the first member of the group and, believing it was a protozoan named it *Duboscqia penetrans* He could not have realized its bacterial nature, mainly because ultrastructural techniques were not available to him and the concept of the prokaryotic cell had not yet been introduced Thorne s (1940) description and nomenclature persisted for 35 years Although other scientists worked with the organism and questioned its taxonomic placement (Williams 1960) it was not until the reexamination by Mankau (1975a), using electron microscopy, that its affinity to bacteria rather than protozoa was revealed The nematode parasite was renamed *Bacillus penetrans* (Thorne, 1940) Mankau, 1975

Only a decade ago Sayre et al (1983) established a sound basis for the taxonomy of this group of hyperparasites Detailed comparisons of the genera *Pasteuria* and *Bacillus* cleared up the confusion and revealed major similarities between the nematode parasite and *Pasteuria ramosa* (Sayre and Wergin 1977 Sayre et al 1983) Their close relationship became obvious in several distinctive morphological characteristics, such as the dichotomously branched mycelial microcolonies that give rise to fragmentation and sporangia, and finally to endogenous spores formed within the old mother cell wall Similarities also had been shown at the ultrastructural level in the unique forms and sequences of life stages of the two organisms (Sayre and Starr 1985). The parasite of plant parasitic nematodes previously known as *Bacillus penetrans*, was then placed in the genus *Pasteuria* and renamed *Pasteuria penetrans* However, the newly named bacterium was by no means a uniform entity Different isolates of this organism were found to differ in their physical and pathological characteristics (Bird et al 1990) The description of two new species followed *Pasteuria thornei* Starr & Sayre (1988) which parasitized the lesion nematode *Pratylenchus brachyurus* (Godfrey) and *Pasteuria nishizawae* Sayre Wergin Schmidt & Starr (1991) which parasitized cyst nematodes, *Globodera rostochiensis* Woll and *Heterodera glycines* Ichinohe A fifth species which parasitized the pea cyst nematode, *Heterodera goettingiana* Liebscher has been reviewed for taxonomic classification (Sturhan et al 1994) Numerous *Pasteuria* spp have endospores distinctly smaller or larger than those previously described (Chen 1996 Ciancio and Mankau 1989 Ciancio et al 1992 1994 Giblin-Davis 1990 Giblin-Davis et al., 1990 Jaffee et al 1985, Noel and Stanger 1994 Sayre et al 1985) Some isolates display a cross-genera host range and various biological characteristics (Bhattacharya and Swarup, 1988 Mankau 1975, Oostendorp et al 1990 Pan et al, 1993 Sharma and Davies 1996 Vargas and Acosta 1990) Thus, *Pasteuria* spp are being differentiated by host specificity,

developmental characteristics, and shape and size of sporangia and endospores (Sayre and Starr, 1989)

Based on a study of host records and endospore morphometrics of pathotypes described previously as the *P. penetrans* group, Ciancio et al. (1994) concluded that host taxonomy and endospore dimensions are of limited value to the definition of *Pasteuria* spp. This would partially explain a discovery by Davies et al. (1992) who identified different surface proteins of endospores in three *P. penetrans* populations all parasites of *Meloidogyne incognita*. A recent study shows that *P. penetrans* produces heterogeneous endospores (Davies et al., 1994). These subpopulations of endospores show specificity to various nematode populations.

No definite criteria or genetic data are available to establish whether distinct species of *Pasteuria* differing in their biology and physiology share common hosts and morphometrics, or whether some or even all of the *Pasteuria* members should be considered as pathotypes of a unique species regardless of hosts and morphometrics (Ciancio et al. 1994). The confusion in taxonomy of *Pasteuria* probably will not be clarified until the bacterial genom properties are elucidated. Axenic cultivation of the bacteria is crucial to understanding the biology and taxonomy of *Pasteuria*, but artificial cultivation of *Pasteuria* spp. has not been successful (Bishop and Ellar, 1991; Previc and Cox, 1993; Reese et al. 1988).

Life Cycle of *Pasteuria* spp.

The life cycle of *P. penetrans* in root-knot nematodes was first described in detail by Sayre and Wergin (1977). Their observations were confirmed by Imbriani and Mankau

(1977) and additional studies have contributed useful information on the life cycle (Davis et al 1988 Mankau and Prasad, 1977 Sayre 1988 Sayre and Starr, 1985 Sturhan et al , 1994) *Pasteuria penetrans* is a density dependent obligate parasite that grows and multiplies within the bodies of plant parasitic nematodes Up to 2 5 million nonmotile endospores are produced per female of *Meloidogyne* spp (Hewlett and Dickson, 1993) and released into the soil environment upon degradation of the nematode carcass

The developmental stages of all members of *Pasteuria* spp parasitic on nematodes appear to be similar but there are differences in shapes and sizes of endospores and sporangia among isolates obtained from different nematode genera. The following detailed description and illustration of the developmental stages covers *P penetrans* and *Meloidogyne* sp and draws attention to other species for notable differences (Fig 1-1)

Attachment. The organism encounters host nematodes when endospores passively adhere to the cuticle of a migratory stage of the nematode in soil. In the case of *Meloidogyne* sp , a single second-stage juvenile may have one to several hundred endospores attached to its cuticle (Davies et al., 1991) Mobility and infectivity of the second-stage juveniles (J2) is reduced when somewhere between 7 and 50 endospores are attached to the juvenile (Brown and Smart 1985 Davies et al 1988 1990 Sell and Hansen 1987 Stirling 1984) Since attachment can occur readily in soil or aqueous suspension (Slana and Sayre 1982) there appear to be no requirements for adhesion other than those found on the endospore or nematode cuticle surface.

There is considerable variation in the ability of *P penetrans* to attach to and infect species of nematodes, particularly within isolates parasitic on *Meloidogyne* spp (Brown and Smart, 1984 Davies et al., 1988 Davies and Danks, 1992 1993, Oostendorp et al , 1990 Sell and Hansen, 1987 Spaull 1984 Stirling 1985 Verdejo-Lucas 1992) Cuticular variations in ultrastructure and composition between nematode populations

Fig. 1–1. Life cycle of the root-knot nematode *Meloidogyne* sp. with and without its bacterial parasite *Pasteuria penetrans* (inner circle). 1) Second-stage juveniles (J2) entering root tip. 2) migrating intercellulary in cortex. 3) J2 establishing feeding sites in the vascular system. germinating *P. penetrans* endospores. 4) third stage juveniles. 5) fourth stage juveniles. 6. 7) young females. 8) female with *P. penetrans* lays no or few eggs whereas a healthy female forms an egg mass and lays eggs. and 9) infected female carcass degrades and releases mature endospores into the soil. The inner circle illustrates the life cycle of *P. penetrans* and its various developmental stages. Cross sections and top views of a) mature endospores. b) endospore attached to cuticle of *Meloidogyne* sp. c) germinating endospore. d) microcolonies formed in the pseudocoelom of the nematode, e) septations in rapidly growing thallus. f) dichotomously branched hyphae with elongated terminal cells. g) fragmented thalli separated from the thallus and visible forespore. h) cell wall separates forespore from parasporum of the egg shaped sporangium. i) differentiation of spore core and parsporal fibers. j) mature endospores surrounded by exosporium and sporangium, and k) endospores released into soil.

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(Davies and Danks, 1992 Reddigan et al , 1986) may be responsible for different levels of attachment. The greatest spore attachment occurs when endospores are exposed to *Meloidogyne* spp. from which they were originally isolated (Davies et al., 1988 Davies et al 1994 Oostendorp et al 1990) Some *P penetrans* isolates attached indiscriminately to *Meloidogyne* spp other than the original host (Stirling, 1985)

The infective juvenile of *Meloidogyne* spp is the only stage that appears to be parasitized by *P penetrans* (Mankau 1980b) and this could perhaps be attributed to the cuticular surface composition differences observed between the nematode s life stages (McClure and Stynes 1988) It has been suggested that the surfaces of J2 contain structural carbohydrate recognition units that are probably not collagen, and that these interact with N-acetylglucosamine moieties on the endospore surface that are linked to either glycoproteins or peptidoglycans (Bird et al. 1989 Davies and Danks 1993 Spiegel et al 1996) However it also was suggested that collagens might be involved in attachment (Persidis et al , 1991) Several studies suggest that host specificity is caused by differences in the amount and nature of surface proteins of the endospores (Chen et al , 1997a Davies et al 1992 1994)

Infection and development. Endospore attachment to J2 of *Meloidogyne* sp is not necessarily followed by infection (De Silva and Gowen, 1994) The endospore-encumbered J2 penetrate the growing roots of a host plant behind the root cap In the zone of cellular differentiation the J2 reside in cortical tissue with their heads in the periphery of the vascular tissue where feeding starts (Hussey 1985) Thereafter a germ tube emerges from a central pore at the basal side of the endospore and penetrates the cuticle and hypodermis of the nematode (Sayre and Starr, 1985) There is evidence that germ tubes which do not penetrate the cuticle emerge between the "rim" of the perisporium and the

cuticle of the nematode (Birchfield and Antonopoulus 1976) Endospore germination appears to be triggered by the onset of feeding (personal observations)

The protoplast of the endospore enters the nematode body through the germ tube and develops into vegetative, spherical colonies consisting of a dichotomously branched, septate mycelium (Sayre and Starr 1985) These early stages (mycelial colonies) are visible with the light microscope as dense granulation within the nematode pseudocoelom. Fragmentation of the thalli separates the mycelial masses, which are then transported in the fluid of the pseudocoelomic cavity through the body of the host nematode These fragments give rise to microcolonies of four eight or more terminal cells In advanced developmental stages, an increasing number of colonies with two three, or four club-shaped enlarged terminal cells are seen (doublets triplets and quartets). These each give rise to a single sporangium in which a single endospore is formed. The process of endospore formation appears typical of that found in other endospore-forming bacteria (Chen et al , 1997b)

As the sporangium enlarges a septum is formed in the upper third of the mother cell separating the incipient forespore cytoplasm from the remainder of the cell (Cheo et al 1997b) The septum growing around the forespore finally provides a double-layered membrane that encloses the condensed cytoplasm The wing-like perisporium and the cortex develop between the two membranes surrounding the forespore An inner cortex and outer zone surrounded by an irregular granular epicortical layer develops The outer spore coat is deposited on the outer membrane and forms the laminar inner coat of the mature endospore Granular material of the spore mother cell concentrates at the basal side of the endospore and is finally engulfed by the exosporium which surrounds the entire endospore Sporangia are of lenticular almost spheroidal shape with a round to conical upper part and an irregularly shaped basal part. When and where the endospores are

released from the sporangia remains unknown. The germination of a single spore is enough to create infection in a *Meloidogyne* sp. female (Stirling 1984). Generally the infection rate of females increases with increasing endospore attachment on the juvenile (Davies et al. 1988, Stirling 1984).

Pasteuria penetrans is morphologically different from *P. thornei* and *P. nishizawae* in shape and size of the sporangia and the endospores (Sayre et al. 1988, 1991). Light microscopy revealed that mature endospores of *P. penetrans*, including parasporal fibers and sporangial wall, are saucer to bowl-shaped, measuring about 4.5 µm in diameter and about 3.6 µm in height. Endospores of *P. nishizawae* are of similar shape with a broadly elliptical central body, averaging 5.3 µm in diameter and about 4.3 µm in height. Endospores of *P. thornei*, however, are rhomboidal shaped and smaller in size averaging 3.5 µm in diameter and 3.1 µm in height. The translucent circular perisporium is easily differentiated from the distinct spore wall. In *P. penetrans* and *P. nishizawae* the spore wall decreases in thickness toward the center of the basal side of the endospore. In *P. penetrans* a morphological discreet area has been observed on the basal side of the endospore, which appears to form the germination pore.

Unlike females, males of *Meloidogyne* spp. seem to become parasitized by *P. penetrans* rather rarely (Abrantes and Volas 1988, Freitas et al. 1996, Hatz and Dickson, 1992, Page and Bridge 1985). Endospores obtained from seven isolates of *P. penetrans* did not attach to males of *M. arenaria* (Freitas et al. 1996) and observations by Hatz and Dickson (1992) suggest that infected males were a consequence of sex reversal because no males with a single gonad were observed to contain endospores.

Some isolates of *P. penetrans* have been reported to complete their life cycles in J2 of *Meloidogyne* spp. (Giblin-Davis et al. 1990, Dickson et al. 1994). Other *Pasteuria* spp. from *Tylenchulus semipenetrans* Cobb and *Heterodera avenae* have endospores that

are morphologically similar to those of *P. penetrans*, but they reproduce in J2 and not in females (Davies et al. 1990, Fattah et al., 1989, Kaplan 1994, Sturhan et al., 1994., Winkelheide 1993). The isolate specific to *T. semipenetrans* also reproduce in the male (Kaplan 1994). Another isolate of *P. penetrans* was able to reproduce in *Pratylenchus scribneri* and *Meloidogyne* spp. (Oostendorp et al., 1990). *Pasteuria* sp. from *Helicotylenchus lobus* formed mature endospores in the juvenile, female, and male life stages (Ciancio et al., 1992). The life cycle of *P. thornei* can be completed in any of the juvenile stages and in the adult of its host *Pratylenchus brachyurus* (Starr and Sayre, 1988).

Ecology of *Pasteuria* spp.

Pasteuria spp. are widespread in different biotopes and habitats (Ciancio et al., 1994). Their spores have the morphological and biochemical features of bacterial endospores (Sayre and Wergin, 1977, Williams et al., 1989) and therefore can tolerate environmental extremes. Factors such as soil moisture, soil temperature, soil pore size, organic matter, and clay content are important in the ecology of *P. penetrans*.

Geographic distribution. *Pasteuria* spp. are worldwide in distribution and have been reported from many countries (Table 1-1).

Moisture level. Endospores of *Pasteuria* spp. are resistant to desiccation (Williams et al., 1989b). The water content of soils, however, affects hatching and movement of nematodes (Baxter and Blake, 1969), and, therefore, is likely to influence the efficacy of *P. penetrans*. Davies et al. (1991) reported soil moisture affects the growth of *P. penetrans* within developing females. However, *P. penetrans* was cultivated successfully in a hydroponic solution (Serracin et al., 1997), which suggests that the failure

TABLE 1-1 Geographic distribution of *Pasteuria* spp

Location	Literature source
North America	
Canada	Gonzales et al. 1987 Sayre and Starr 1988 Sturhan 1988
U.S.A.	Allen 1941 Altherr and Debouteville 1972 Bernard and Niblack 1982 Ciancio et al., 1994 Mantoa and Sayre 1989 Sayre and Starr, 1988 Sturhan 1988
U.S.A., California	Ciancio et al.,1992 Ciancio and Mankau 1989b Sayre and Starr 1988
U.S.A., Colorado	Sayre and Starr, 1988
U.S.A., Florida	Chen 1996 Esser 1980 Giblin-Davis et al. 1990 Hewlett et al., 1994 Inserra et al., 1992 Kaplan 1994 Oostendorp et al., 1990 Sayre and Starr 1988 Walter and Kaplan 1990 Weibelzahl-Fulton et al., 1996
U.S.A., Georgia	Sayre and Starr, 1988 Minton and Sayre, 1989
U.S.A., Hawai	Ko 1995 Sayre and Starr 1988
U.S.A., Illinois	Noel and Stanger 1994
U.S.A. Louisiana	Sayre and Starr 1988
U.S.A., Maryland	Sayre and Starr, 1988
U.S.A., Oregon	Sayre and Starr, 1988
U.S.A., South Carolina	Sayre and Starr, 1988
U.S.A. South Dakota	Sayre and Starr, 1988
U.S.A., Utah	Sayre and Starr, 1988
Central America	
Colombia	Ciancio and Mankau 1989a
Cuba	Sturhan, 1988
Dominican Republic	Sayre and Starr 1988
Haiti	Sayre and Starr, 1988
Nicaragua	Sayre and Starr, 1988 Sturhan 1985 1988
Puerto Rico	Vargas and Acosta, 1990 Vargas et al., 1992
South America	
Bolivia	Ciancio and Mankau, 1989b, Page and Bridge 1985 Sturhan 1988
Brazil	Dos Santos, 1981 Sayre and Starr 1988, Sturhan, 1988
Peru	Ciancio and Mankau 1989a Ciancio et al., 1994
Venezuela	Sayre and Starr 1988
Europe	
Austria	Sayre and Starr, 1988
Belgium	Sayre and Starr, 1988
Croatia	Ciancio et al., 1994
Denmark	Williams 1960
England	Davies et al., 1990 Sturhan 1988
Finland	Sayre and Starr 1988
France	Sturhan 1985 1988
Germany	Sayre and Starr 1988 Sturhan 1988, 1985 Stemer 1938
Greece	Vovlas et al., 1993 Sayre and Starr 1988
Hungary	Ciancio et al., 1994
Iceland	Sayre and Starr 1988 Sturhan 1988
Italy	Abrantes and Vovlas 1988 Ciancio 1995 Ciancio et al., 1987 1994 Davies et al., 1990 Roccuzzo and Ciancio, 1992, Walia et al., 1990

TABLE 1.1. Continued

The Netherlands	Kuiper 1958, Sayre and Starr 1988
Poland	Ciancio et al. 1994
Portugal	Abrantes and Vovlas 1988
Romania	Ciancio et al. 1994, Sayre and Starr 1988
Scotland	Sayre and Starr 1988
Spain	Verdejo Lucas 1992
Sweden	Sayre and Starr 1988
Switzerland	Sayre and Starr 1988, Sturhan 1988
Africa	
Algeria	Ciancio et al. 1994
Ethiopia	Ciancio et al., 1994
Ivory Coast	Sturhan 1988
Liberia	Ciancio et al. 1994
Malawi	Sturhan 1988
Mozambique	Sturhan 1988, Sayre and Starr 1988
Nigeria	Sayre and Starr 1988, Sturhan 1988
Senegal	Mankau 1980
Sierra Leone	Ciancio et al. 1994, Sayre and Starr 1988
Somalia	Ciancio et al. 1994
South Africa	Sayre and Starr 1988, Spaull, 1981, Sturhan 1988
Tanzania	Madulu et al. 1994, Siddiqi 1991
Togo	Sayre and Starr 1988
Uganda	Sayre and Starr 1988
Zaire	Sayre and Starr 1988
Zimbabwe	Stubbs and Gowen 1996
Asia	
China	Pan et al. 1993
India	Bhattacharya and Swarup 1988, Page and Bridge, 1986, Sharma and Davies 1996, Sharma and Sharma, 1989, Walia et al. 1990
Iran	Barooti 1989, Maafi 1993, Sayre and Starr, 1988, Sturhan, 1988
Iraq	Fattah et al. 1989
Israel	Ciancio et al. 1994, Sayre and Starr 1988
Japan	Sayre and Starr 1988, Sayre et al. 1991a, Sturhan 1988
Pakistan	Maqbool and Zaki 1990
South Korea	Ciancio et al., 1994
Sri Lanka	Ciancio et al. 1994
U S S R	Sturhan 1988, Sabbotin et al. 1994
Australia	
South Australia	Bird and Brisbane 1988, Sayre and Starr 1988, Stirling and White 1982, Sturhan 1988
Islands	
Azores	Sayre and Starr 1988, Sturhan 1988
Canary Islands	Sayre and Starr 1988, Sturhan 1988
Madeira Islands	Sayre and Starr 1988
Malta	Ciancio et al. 1994
Mauritius	Ciancio et al. 1994, Sayre and Starr 1988, Williams 1960
Philippines	Ciancio et al. 1994, Sturhan 1988
Samoa	Sayre and Starr 1988
São Tomé	Ciancio et al. 1994

of Davies' system was probably due to poor development of the root system in wet soils. Isolates of *P. penetrans* have survived for several weeks in dry, moist, and wet soils, and in soils with fluctuating moisture levels without loss of their ability to attach to their nematode hosts (Oostendorp et al. 1990). Their abundance in soils is positively correlated with increasing amounts of annual rainfall (Ko et al., 1995).

Temperature. Attachment, infection, and pathogenesis of *Pasteuria* spp. is affected by temperature (Ahmed and Gowen, 1991; Freitas et al., 1997; Hatz and Dickson, 1992; Nakasono et al. 1993; Sekhar and Gill 1990a; Serracin et al. 1997; Serracin Ulate 1995; Singh et al. 1990; Stirling 1981; Stirling et al., 1979, 1990). Stirling (1981) found that temperatures near or above the optimum for the nematode caused *P. penetrans* to attach at higher rates and to proliferate extensively before the infected female host reached maturity. In contrast, at 20 °C the rate of attachment decreased and females often developed ovaries containing eggs before infection prevented further development.

Endospore densities of *P. penetrans* in soil increase with an increase of the average annual temperature (Ko et al., 1995). Walker and Wachtel (1988) observed that soil solarization with clear polyethylene increased the rate of infection of *Meloidogyne javanica* by *P. penetrans*. The higher soil temperature may have led to an increase in attachment rates because of the likely increase in nematode mobility, thus leading to an increased probability of endospore contact with J2, on the other hand the higher temperature may have simply increased the rate of nematode development, thereby increasing endospore numbers in soil. Recent studies however have shown that attachment of endospores to J2 was reduced after endospores were exposed to 40, 50, and 60 °C (Freitas et al., 1997). Attachment occurred following heating of endospores to 80 °C for 30 minutes, but *P. penetrans* did not develop inside the nematode (Dutky and Sayre 1978). Although the rate of attachment has been reported to be reduced when endospores were treated at 100 °C for

at least 30 minutes attachment was not prevented (Freitas, 1997, Stirling et al., 1986, Williams et al., 1989b). The greatest receptivity of J2 of *M. arenaria* to endospore attachment occurred when J2 were treated in water at 30 °C and 35 °C and then exposed to endospores (Freitas et al., 1997).

Soil type. *Pasteuria penetrans* was detected in a wide variety of soil types ranging from pure sand to organic soils (Sturhan, 1985). Observations in laboratory experiments suggest that an increasing sand content might improve endospore attachment (Singh and Dhawan, 1992). Other workers demonstrated that soil texture had no effect on endospore attachment (Hewlett, personal communication). Sandy soils allow endospores to distribute readily with percolating water (Oostendorp et al., 1990).

Organic matter. The organic matter component of soil is unlikely to influence *Pasteuria* spp., other than providing fungal antagonists of nematodes with more favorable habitat and food source (Kerry, 1993). Habitat and food sources may influence the biological control attributes of *P. penetrans* (Dube and Smart, 1987; Maheswari and Mani, 1988). Mode of action of organic amendments against nematodes consists of more than the direct effects on nematophagous fungi. When organic soil amendments in the form of oil cakes were added to *P. penetrans* infested soil in a pot experiment, *M. javanica* was synergistically reduced (Maheswari et al., 1988). Organic amendments can improve soil structure and soil fertility, alter the level of plant resistance, release toxic compounds, and stimulate nematode antagonistic microorganisms (Stirling, 1991). All of these changes by organic amendments may affect soil nematode densities dramatically.

Chemical factors. *Pasteuria penetrans* was detected in acidic as well as in alkaline soils (Sturhan, 1985). However, the optimum pH for attachment of endospores of *Pasteuria* spp. is between 7.0 and 8.5 (O Brian, 1980). The endospore's resistance to chemical compounds allows them to survive nematicide applications (Mankau and

Prassad, 1972 Stirling 1984) Nematostatic field dosages of 1,3-dichloropropene aldicarb carbofuran, fenamiphos, and ethoprop had no noticeable effect on *P penetrans* (Mankau and Prassad 1972) Increased attachment by *P penetrans* was observed when subnematostatic concentrations of organophosphate or organocarbamate nematicides were applied to soil containing nematodes and endospores (Brown and Nordmeyer 1985) As reported by Bunt (1987) the low concentration of a nematicide might have increased the random nematode movement in the soil which would increase the likelihood of contact with bacterial endospores Freitas (1997) reported that chloropicrin alone or in combination with methyl bromide was highly detrimental to the development of *P penetrans* because endospore formation was inhibited

Host/parasite relationship. *Pasteuria penetrans* parasitism has been recorded for more than 236 nematode species in 102 genera including plant parasitic and free living species (Ciancio et al., 1984 Sturhan 1985 1988) In several observations the host parasite relationship is based on cuticular attachment only Stirling (1991) concluded that such published data should be treated with caution because endospore attachment does not actually determine parasitism. Because of its obligate nature the bacterium is unable to reproduce in the absence of the host and would, therefore be disadvantaged if it eliminated its host With increasing endospore densities in soils the infectivity of endospore encumbered J2 of *Meloidogyne* sp has been shown to be reduced thus leading to the production of fewer endospore-filled females (Stirling 1991) Endospore densities are likely to stabilize at a certain equilibrium level (Stirling, 1991) Williams (1960) and later Spaull (1984) noted this density dependent relationship between *P penetrans* and its *Meloidogyne* host

Biological Control of Nematodes by *Pasteuria* spp.

Biological control attributes

Since the time that Sayre and Starr (1988) reported *Pasteuria* spp. to be parasitic to most species of plant parasitic nematodes, much effort has been made to understand *P. penetrans* isolates that parasitize the economically important root knot nematodes. *Pasteuria penetrans* has many of the attributes required by a successful biological control agent. The bacterium produces no environmental hazards and reduces or prevents reproduction of its host. Also, it reduces the infectivity of endospore-encumbered juveniles to their plant host. Infectivity may be reduced when as few as 15 endospores are attached to the juvenile (Davies et al. 1988). Juveniles are prevented from invading roots when they are each encumbered with 25 to 30 endospores (Stirling 1984; Stirling et al. 1990).

Host specificity. Isolates of *P. penetrans* can vary in specificity to different *Meloidogyne* spp. (Brown and Smart, 1984; Davies et al. 1988; Davies and Danks 1992, 1993; Oostendorp et al. 1990; Sell and Hansen, 1987; Spaull 1984; Stirling 1985; Verdejo-Lucas 1992). Field populations of *Meloidogyne* spp. may be diverse, and it is possible that populations of *P. penetrans* with a restricted host range will affect nematode species to varying degrees. However, if there is time for natural selection to take place, it may be possible for new strains of *Pasteuria* spp. to evolve that are better fitted to the local environment or that are more virulent to the local nematode population. Davies et al. (1988) and Channer and Gowen (1992) support this concept by demonstrating that an initially poor host of a *P. penetrans* isolate became more susceptible to the parasite when exposed to endospores grown on nematodes of its own species. However, individual nematodes escaping parasitism produced a generation with increased resistance to attachment by endospores of that particular *P. penetrans* isolate. The authors concluded

that populations of *P. penetrans* and *Meloidogyne* spp. can be genetically heterogeneous with respect to host specificity and susceptibility respectively. Thus, an isolate of *P. penetrans* with a wide host range of plant-parasitic nematodes needs to be employed to ensure durable biological control (Channer and Gowen, 1992, Tzortzakakis and Gowen, 1994b). Genetic diversity within a population of *P. penetrans* may enable the organism to maintain itself in situations in which nematode populations are altered by a change in cropping practice (Stirling, 1991).

Environmental factors. Endospores can be stored in air-dried soil and root material for up to several years without apparent loss in the ability to attach to host nematodes (Mani, 1988, Mankau and Prassad, 1977, Stirling and Wachtel, 1980). Their viability was retained following repeated freezing and thawing (Bird et al., 1990). Since the endospores are relatively small, they can be applied easily to the soil surface and redistributed by percolating water (Oostendorp et al., 1990), which is a particularly useful attribute for a biological control agent (Stirling, 1991).

Obligate parasitism. The apparent obligate nature of parasitism by *Pasteuria* spp. limits their biological control potential for two reasons. Spores that lose their viability or are consumed by other soil organisms can only be replaced in the presence of host nematodes and susceptible host plants. More problematic is the lack of efficient technology for the industrial production of the organism (Bishop, 1991, Dickson et al., 1994, Reise et al., 1988, Stirling, 1991). This constraint stimulates efforts in the exploitation of naturally occurring isolates of *P. penetrans* for control of *Meloidogyne* sp. in home gardens, small-holdings of subsistence farmers (Gowen and Ahmed, 1990), and horticultural industries (Dunn, personal communication, Giblin-Davis, 1990).

Natural control

Plant-parasitic nematodes and their bacterial parasites have co-evolved for a long time (Cianeio et al., 1994). There are few data on the extent of nematode suppression caused by *Pasteuria* sp. in undisturbed soil or in soils with continuing monoculture. With increasing awareness about the qualities of the hyperparasites, more effort have been invested in long-term field studies, and the processes in which soils become suppressive to nematodes are becoming better understood (Chen, 1996b, 1997c, Dickson et al., 1994, Oostendorp et al., 1991a, Stirling, 1991, Weibelzahl-Fulton et al., 1996).

Pasteuria sp. may have little short-term impact on nematode populations at low spore densities because only a few nematodes come in contact with the endospores. However, with each infected root-knot nematode female capable of producing up to 2.5 million endospores, endospore densities increase over time, thereby reaching nematode suppressive levels (Oostendorp et al., 1991a, Chapter 3). Williams (1960) and later Spaull (1984) noted that *P. penetrans* had little impact on the severity of root-knot nematodes in a natural infestation of both parasites; however, the bacterial parasite may still have been in an early phase of population build-up. Others have reported soils with root-knot nematodes and *P. penetrans* becoming highly suppressive to the nematode (Bird and Brisbane, 1988, Chen et al., 1994, Dickson et al., 1994, Minton and Sayre, 1989, Stirling, 1984, Weibelzahl-Fulton and Dickson, 1996, Weibelzahl-Fulton et al., 1996). When root-knot nematode and *P. penetrans* infested soils were tested for their suppressiveness, the rate of reproduction of *Meloidogyne* spp. was frequently reduced in untreated soils compared to soils in which *P. penetrans* had been eliminated by autoclaving (Dickson et al., 1994, Weibelzahl-Fulton et al., 1996). Nematode suppression was enhanced by cultivation of susceptible hosts for several consecutive years.

In a pot experiment, soil naturally infested with *Belonolaimus longicaudatus* Rau and *a Pasteuria* species specific to the nematode was not suppressive to its host nematode for the first 6 months (Giblin-Davis et al. 1990) however, at 12 months post-inoculation there was an increase in the number of *Pasteuria*-filled nematodes The nematode density was significantly lowered (Giblin Davis 1990)

Inundative application

Despite the difficulties scientists originally faced in working with spore-infested soil *P. penetrans* gave promising results against *M. incognita* in pot experiments (Mankau, 1975b) An improvement of the mass production technique allowed scientists to produce a spore laden, easily handled root powder (Stirling and Wachtel 1980) This provides a means for more extensive testing and further confirmation of the bacterium s potential as a biological control agent. Control of *Meloidogyne* sp. by *P. penetrans* was reported from greenhouse tests (Brown and Nordmeyer 1985 Channer and Gowen 1988 Maheswari and Mani 1988 Maheswari et al 1987 1988 Raj et al , 1988 Raj and Mani, 1988 Tzortzakakis..1994a Vargas et al ..1992), microplot studies (Brown et al , 1985 Chen et al ..1996b..1997c; Daudi 1990, Dube and Smart, 1987, Oostendorp et al 1991a), and small scale field experiments (Channer and Gowen, 1988, Stirling, 1984)

Several investigators have provided estimates of the endospore population density needed in the soil to provide nematode control (Brown and Smart, 1985 Chen et al , 1996b Davies et al. 1989 1990; Sell and Hansen, 1987 Stirling 1984) A concentration of 10^3 endnspores/g of soil prevented J2 of *Meloidogyne* spp from infecting plant roots when the nematode moved either 4 or 8 cm through the soil (Stirling et al , 1990) Nematodes that moved 2 cm through the soil were infective but they did not produce progeny because of infection by the bacterial parasite Concentrations as low as 10^2

endospores/g of soil reduced root knot nematode fecundity (Ahmed and Gowen 1991, Gowen et al 1989) and caused suppression of *M arenaria* infection on peanut (Chen et al 1996b) Densities of 10^5 endospores/g of soil throughout the top 15 cm provides levels of control comparable to a nematicide application (Stirling, 1991). Most recently Chen et al (1996b) demonstrated that adding 10,000 to 100 000 endospores per g of soil to root knot nematode infested soil provided control of *M arenaria* on peanut in the first season Application of *P penetrans* endospores 2.5 cm deep in soil appeared to be more effective for parasitism of *M incognita* on tomato than a surface application or an application 5 cm deep (Ahmad et al., 1994)

Integrated nematode management

There have been several attempts to integrate *P penetrans* with other nematode management techniques. Synergistic effects on the management of *Meloidogyne* spp were reported from greenhouse experiments in which *P penetrans* was combined with organophosphate or organocarbamate nematicides at nematostatic field application rates (Maheswari et al 1987 Tzortzakakis, 1994a) or at subnematostatic rates as low as 1 5 ppm and 0 25 ppm, respectively (Brown and Nordmeyer 1985) Successful control of *Meloidogyne* spp also may be obtained by combining *P penetrans* with one or several other biological control strategies. De Leij et al (1992) noted that the egg mass-colonizing fungal endoparasite *Verticillium chlamydosporium* Goddard complimented the suppression of *M incognita* by *P penetrans* by attacking a different life stage of the nematode thus giving better results than either of the organisms alone or the nematicide treatment. Similar results were obtained in experiments where *Paecilomyces lilacinus* (Dube and Smart, 1987, Maheswari and Mani, 1988 Shahzad et al 1990 Zaki and Maqbool, 1991)

Talaromyces flavus or *Bacillus subtilis* (Zaki 1991) were added as additional nematode antagonists or where oil cakes were added as a soil amendment (Maheswari et al. 1988)

The efficacy of *P. penetrans* was enhanced with soil solarization applied alone (Freitas 1997, Walker and Wachtel 1989) and in combination with an organophosphate nematicide (Tzortzakakis 1994a, Walker and Wachtel 1989). The population density of *P. penetrans* also was affected by crop rotations and winter cover crops. In the presence of a susceptible host plant, the density of *P. penetrans* endospores build up in the presence of an increasing *Meloidogyne* spp. population (Brown et al., 1985, Chen et al., 1994, Madulu et al., 1994, Oostendorp et al., 1991a). A non-host, resistant host, or fallow rotation has been shown to hamper the increase in endospore density (Chen et al., 1994, Madulu et al., 1994, Oostendorp et al., 1991a). Summer crops were generally more effective than winter cover crops, which is probably explained by the high temperature dependence of both the root-knot nematode and its bacterial parasite.

Useful Methods and Techniques for Studying *Pasteuria* spp.

Detection of *Pasteuria* spp. in soils and in nematodes

Pasteuria spp. can be detected readily on or in nematodes using a number of simple techniques. Endospore-encumbered or endospore-filled migratory nematode stages have to be extracted from the soil. One method for their extraction is to use a centrifugal flotation technique (Jenkins, 1964). Increasing the specific gravity of the sucrose solution used in the centrifugal-flotation technique from 1.14 to 1.22 and 1.26 led to recovery of a higher number of spore filled bodies of *Pratylenchus scribneri* and *B. longicaudatus* respectively (Oostendorp et al., 1991b). Extraction efficacy of endospore-filled *Hoplolaimus galeatus* (Cobb) Thorne was not increased with the denser sucrose solutions

Nematodes may be examined for adhering or internal endospores with the aid of an inverted microscope at ×400 magnification. For easier detection, endospores adhering to the body of a nematode may be stained with Brilliant Blue G (Bird, 1988) or labeled with fluorescein (Charnecki 1997, Charnecki et al 1996). Detection of endospores in soil can be increased by adding host nematodes to a soil sample and storing the sample for several days to allow the nematodes to migrate through the soil, or by adding the nematodes and water to the soil and shaking the contents for 24 hours before extraction (Stirling and White 1982). The attachment of endospores to the nematode body does not indicate infection, evidence of internal parasitism is needed to confirm that the nematode is actually a host. The parasite's presence can be confirmed by squashing females on a glass slide and observing them for the presence of vegetative stages or endospores of the parasite (Hatz and Dickson, 1992). For better recognition of the different developmental stages of the bacterium, specimens can be heat fixed and Gram stained (Cappuccino and Sherman, 1986). A simpler technique by Serracin et al (1997) used lactophenol and 1% methyl blue stain to dye the bacterial structures inside the nematode body. However, its application did not successfully stain bacterial structures in vermiform J2 of *Meloidogyne* sp.

Isolation of *Pasteuria* spp. from soils and nematodes

With the exception of the soil-inhabiting mature endospore, the bacterium's other life stages can only be found inside nematode bodies. In sedentary nematodes, such as *Meloidogyne* spp., *Tylenchulus semipenetrans*, *Heterodera glycines*, *H. goettingiana* and *Globodera rostochiensis*, early life stages of *Pasteuria* spp. can be found in sedentary juvenile stages and in the adult female (Sayre et al., 1991a, Sturhan et al. 1994, Oostendorp personal communication). These nematode life stages can be dissected from enzymatically digested plant tissue (Hussey 1971). Endospore-filled females of

Meloidogyne spp are usually white, opaque and larger than uninfected females (Hatz and Dickson, 1992). The infected females can be distinguished visually and collected by hand with the use of a stereomicroscope. Migratory life stages of sedentary nematodes and ectoparasitic nematodes parasitized with *Pasteuria* spp. must be hand-picked from nematode suspensions after they are extracted from soil. By surface sterilizing infected nematodes and squashing them in sterilized water, different life stages of *Pasteuria* spp can be observed free of contaminating microorganisms (Williams et al., 1989).

Quantification of endospores in soils, root powder, or suspensions

The quantification of the bacterial endospores is important, because the efficacy of *Pasteuria* spp is density dependent (Stirling, 1991). However, methods for enumeration of *Pasteuria* spp in soils have received little attention, and endospore densities in the soil are usually based on the number of endospores attached to soil inhibiting nematode stages. Time and effort to determine the number of endospores of *P. penetrans* attached to J2 of *Meloidogyne* spp may be reduced considerably by using tally thresholds (Chen and Dickson, 1997).

Juveniles of *M. javanica* were used as probes for *P. penetrans* (Stirling, 1984; Stirling and White, 1982) and since there is a direct relationship between endospore concentration in soil and the number of endospores attached (Stirling et al., 1990), a bioassay was developed to estimate the relative endospore density in soil (Oostendorp et al., 1991a). By using a particular probe nematode in the bioassay, only that component of the *P. penetrans* population that attaches to that nematode is detected.

Endospores also have been extracted from soil by differential-centrifugation (Davies et al., 1990); however, the technique is tedious and difficult to use. The total number of endospores in a particular soil may not accurately represent the suppressiveness

of the soil, thus according to Stirling (1991) a bioassay is a more useful quantification method than the use of a direct extraction method

Chen et al. (1996a) observed that the highest estimate of the number of endospores in root material was obtained by suspending machine ground root powder in deionized water and pouring the material onto a 250-µm pore sieve. Endospores passed through the sieve and were concentrated in water, and the number of endospores was calculated with the help of a hemacytometer. This refined quantification allowed an improvement compared to the previous application of the inoculum by weight (Brown et al. 1985; Dube and Smart, 1987; Raj and Mani, 1988; Stirling 1984; Zaki and Maqbool, 1991; 1992a; 1992b)

Culture and preservation of *Pasteuria penetrans*

Methods have been developed for the production of *P. penetrans* endospores in females of *Meloidogyne* spp. parasitizing plant roots. This is currently necessary because *in vitro* cultivation has not been successful (Bishop and Ellar, 1991; Previc and Cox, 1993; Reise et al., 1988). *Pasteuria penetrans*-encumbered J2 of *Meloidogyne* sp. must be inoculated onto a susceptible host, such as tomato (Stirling and Wachtel, 1980). Once the bacterium has developed to maturity, the root systems may be harvested, washed, air dried and ground into a fine endospore laden powder. Although tomato plants are most commonly used, cucumber, *Cucurbita vulgaris* L., was shown to yield higher endospore numbers than tomato (Cho et al., 1997). This system may produce a highly variable yield of endospores; however, it is superior to the use of endospore infested soil (Dutky and Sayre, 1978; Mankau and Prassad, 1977). The plant system has been optimized (Sharma and Stirling, 1991) and most recently a hydroponic cultivation system has been reported (Serracin Ulate, 1995). Verdejo and Jaffee (1988) used a gnotobiotic technique for

producing endospores- however this method requires rigorous aseptic conditions and is therefore not a good procedure for general use

The time period required for *P. penetrans* to complete its life cycle has been shown to vary with different isolates (Hatz and Dickson, 1993, Serracin et al. 1997, Stirling, 1981). Thus, a random determination of the progress of the bacterium's development in a cultivation system can help avoid harvesting prematurely, thus improving the efficiency of the endospore yield. The lack of an efficient technology for the mass-cultivation of *Pasteuria* spp. endospores is the greatest impediment to the use of this microbe as a biological control agent (Dickson et al., 1994, Stirling, 1991).

Attachment

Endospore host attachment studies are the first step in establishing the host specificity of *Pasteuria* spp. and determining their biological efficacies. Most of the techniques rely on nematode movement through soil (Brown and Smart, 1985), water (Channer and Gowen, 1988) or agar (Verdejo and Jaffee, 1988) when each is laden with endospores, or the agitation of a nematode-endospore water suspension (Ahmed et al., 1990, Bird, 1986, Bird et al., 1990; Davies et al., 1988, Oostendorp et al., 1990). Hewlett and Dickson (1993) consistently achieved attachment of *P. penetrans* to *Meloidogyne* spp., *H. galeatus* and *B. longicaudatus* using a centrifuge technique. The method is fast, and allows studies of *Pasteuria* spp. collected from nematode species that yield relatively few endospores per cadaver.

Objectives

The objectives of this dissertation were 1) to monitor the population densities of *Meloidogyne* spp. and *P. penetrans* in microplots and in a naturally infested tobacco field, 2) to develop a test for soil suppressiveness that allowed the determination of the role of *P. penetrans* in suppressing *Meloidogyne* spp. populations in both microplots and tobacco field plots and 3) to evaluate the effect of cultural practices such as an intercropping system with corn and beans in rotation with peanut, autumn cover crops, resistance and fertilizer regimes on the population development of both *M. arenaria* and *P. penetrans*.

CHAPTER 2
POPULATION DEVELOPMENT OF *MELOIDOGYNE ARENARIA* RACE 1 AND *PASTEURIA PENETRANS* IN A 6 5 YEAR MICROPLOT STUDY

Introduction

The peanut root-knot nematode *Meloidogyne arenaria* (Neal) Chitwood race 1, is one of the most important soil pathogens of commercially grown peanut (*Arachis hypogaea* L.) (Minton 1984). It occurs on peanut in many countries and is especially troublesome in the southeastern United States (Minton, 1984; Porter et al., 1984). Continuing environmental problems associated with the use of nematicides (Thomason, 1987) has resulted in more scientists studying nematode management strategies alternative to chemical control (Kerry, 1990). The use of microbial agents for biological control of plant parasitic nematodes is an alternative management tactic that is receiving increased interest among nematologists. Among many organisms identified as antagonists of plant parasitic nematodes, the endospore forming bacterium, *Pasteuria penetrans*, has been demonstrated to have potential for the control of mainly *Meloidogyne* spp. It has been suggested that *P. penetrans* may suppress nematode population densities below economic damage levels (Chen et al. 1996b, Dickson et al., 1994; Minton and Sayre, 1989; Stirling, 1991). In order to determine the efficacy of the density-dependent obligate parasite, it is important to understand its development as influenced by its nematode host.

The population densities of *P. penetrans* and of *M. arenaria* race 1 with and without the nematode antagonist were determined from the spring of 1987 to the fall of

1989 (Oosteudorp et al., 1991a). During this period, soil suppressive to *M. arenaria* was produced in microplots. The microplots had been planted continuously to peanut in summer and either vetch (*Vicia villosa* Roth) or wheat (*Triticum aveuae* L. cultivar 302), rye (*Secale cerale* L. cultivar Wrens Abruzzi), or bare fallowed during the winter. *Pasteuria penetrans*, initially applied to the soil in relatively low numbers, was increased to numbers which suppressed *M. arenaria*. Within 3 years, yield of plots infected with *P. penetrans* and *M. arenaria* increased to a level comparable to the untreated control (Oostendorp et al., 1991a). The objective of this study was to continue to monitor the population densities of both *M. arenaria* race 1 and *P. penetrans* in these microplots in order to determine their long term effects on *M. arenaria* densities.

Materials and Methods

In 1987, 90 microplots (76 cm in diameter), located at the University of Florida, Green Acres Agronomy Farm, Alachua County, were initially arranged in 10 rows of nine plots each, with a distance of 1.5 m between plots, in a loamy, siliceous, hypothermic Grossarenic Paleudults with 90% sand, 4% silt, 6% clay, and 1.8% organic matter (Oosteodorp et al., 1991a). A split-plot design was used with main plots consisting of three soil treatments: an untreated control, *M. arenaria* alone (RKN), and *M. arenaria* plus *P. penetrans* (RKN + Pp). Three summer-winter cover crop rotations of peanut-rye, peanut-vetch through 1991, with peanut-wheat thereafter, and peanut-bare fallow were subplots. Wheat replaced vetch because the incidence of soilborne diseases on peanut increased following vetch. Each combination of soil treatments and winter cover crops was replicated 10 times. The microplots were initiated by placing 800 cm^3 of soil preparation containing uninfected tomato roots; tomato roots infected with *M. arenaria*

race 1, or with *M. arenaria* race 1 plus *P. penetrans* in a hole in the center of each microplot receiving the control, RKN or RKN + Pp treatment, respectively. Monitoring of the bacterium and nematode population densities for this dissertation began in the spring of 1990 and continued through the fall of 1993. Data from the period 1987 to 1989 are included herein to provide a complete data set on the long-term effects of *P. penetrans*.

Two months before planting in the spring of 1991 and 1993, uninoculated control plots were treated with 977 kg methyl bromide/hectare (98% methyl bromide plus 2% chloropicrin) applied broadcast under a 3 mm polyethylene plastic covering. On 6 May 1990, 5 May 1991, 13 May 1992, and 7 May 1993, three pairs of peanut seeds of cv. Florunner were planted 4-cm deep in an equally spaced pattern in each plot. After emergence, one of each pair of seedlings was removed. A 90-cm high, wire-mesh fence was placed around each plot to confine the growth of the peanut foliage inside the microplot. Plots were weeded manually. Through 1991, plots were irrigated by overhead sprinklers. Thereafter, a microjet sprinkler system was installed, delivering an amount of water equivalent to 4 mm per day. Every 10 to 14 days, insects and foliar disease pathogens were controlled with esfenvalerate, chloropyrifos, insecticidal soap, chlorothalonil, or liquid sulfur.

In mid-November each year, after harvest of peanut, rye and vetch or wheat were broadcast seeded as winter cover crops, or plots were bare fallowed through the winter. Vetch, wheat, and peanut are hosts for *M. arenaria* race 1, but rye is a poor host. The soil in each plot was turned using a spade, and leveled between crops.

At harvest (136 to 140 days after planting), peanut plant shoots were evaluated in their appearance, and the root systems, as well as the pods and pegs, were rated for galling. A good stand of plants with a densely closed canopy was rated as 0, and a poor stand of plants with sparse canopy was rated as 5. Galling was rated according to the following

scale 0 = no galls 1 = 1 10 2 = 11 20 3 = 21 55 4 = 56-80 and 5 = 81 100% of roots or pods and pegs galled (Barker et al 1986) Three to five days after the plants were lifted ont of the ground all pods were removed and placed into paper bags dried at 60 C uotil the moisture content was reduced to about 10% and weighed to determine yield The ounber of second stage juveniles (J2) of *M. arenaria* /100 cm^3 of soil was estimated at harvest of peanut and each of the winter cover crops Soil samples consisting of a composite of five 2 5-cm-diameter cores per plot were taken from the top 20 cm of each microplot with the nse of a cone shaped auger The soil was mixed and processed with a centrifugal-flotation method (Jenkins 1964) The nnmbers of J2 the rate of attachment of *P. penetrans* and the nnmber of endospores attached per 20 randomly selected J2 was determined using an inverted microscope at ×400 magnification.

Data were subjected to analysis of variance (ANOVA) If the ntained probability level of the main plot (treatments) or the subplot (cover crop) effects was significant at $P \leq 0.05$ the means were separated by Duncan's multiple range test If a significant main plot × subplot interaction ($P \leq 0.05$) was observed main plot means for each subplot and subplot means for each main plot were separated by Dnncan's multiple range test If an insufficient nnmber of J2 or no J2 were extracted from soil of RKN + Pp plots replicates without J2 were disregarded for the calculation of rate of attachment with *P. penetrans* and the nnmber of endospores per jnvenile and data were presented in a separate table

Results

After the initial 3 year period nf the experiment (1987 to 1989) the nnmber of J2 in the soil of RKN + Pp plots decreased and remained lower than those in the RKN plots ($P \leq 0.05$) (Fig 2 1 Tables 2-t to 2 3) The nematode popnlntion density in microplots

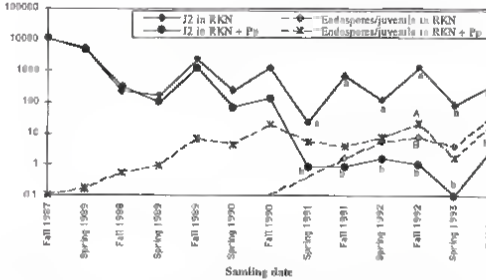


Fig 2-1 Number of second-stage juveniles (J2) of *Meloidogyne arenaria* race 1 in 100 cm^3 of soil and the number of endospores attached per juvenile extracted from peanut microplots infested with *M. arenaria* (RKN) and *M. arenaria* plus *P. penetrans* (RKN + Pp). Analysis was based on means of 10 replicates with 20 or fewer J2 observed per replication to determine the number of endospores per juvenile. Means labeled with a different letter are different according to Duncan's multiple-range test ($P \leq 0.05$); small letters were used for comparisons of juvenile numbers; capital letters for comparing endospore numbers. Data from 1987 to 1989 were from Oostendorp et al. (1991a).

TABLE 2-1 Effect of *Meloidogyne arenaria* (RKN) alone and in combination with *Pasteuria penetrans* (RKN + Pp) and of a rye, vetch, or bare fallow winter cover crop on yield and performance of peanut in the 5th year (fall of 1991) of a 6.5-year microplot experiment.

		J2 per 100 cm³ of soil	% J2 with attached endospores	Endospores per juvenile	Plant performance: Yield (kg/plot)	Plant performance: Sheets[a]	Galling index[b]: Roots	Galling index[b]: Pods
Main effects								
Treatment	Control	1 b	0 b	0 b	269 a	2.9 a	0 b	0 b
	RKN	677 a	55 a	1.6 a	167 b	3.1 b	2.8 a	2.6 a
	RKN + Pp	1 b	23 a	3.7 a	252 a	2.9 a	0.2 b	0 b
Cover crop	Rye	188	17	0.3 b	253	2.7	0.8	0.7
	Vetch	253	25	0.1 b	224	2.6	1.2	0.9
	Fallow	237	36	4.0 a	212	2.5	1.1	1
Interactions								
Rye	Control	0	0	0 b	281	2.5	0	0
	RKN	564	40	0.7 a	193	2.1	2.5	2.1
	RKN + Pp	1	10[c]	0.1[c] a	285	3.5	0	0
Vetch	Control	2	0	0 b	286	3.3	0	0
	RKN	757	76	3 a	133	1.9	3.2	2.6
	RKN + Pp	0	0[c]	0 b	254	2.6	0.3	0
Fallow	Control	1	0	0 c	241	2.9	0	0
	RKN	709	48	1.1 b	176	2.2	3	3
	RKN + Pp	2	60[c]	11[c] a	218	2.5	0.4	0

Means followed by no letters or the same letter within a column of three observations for main effect or 10 observations for interactions were not different according to Duncan's multiple range test ($P \leq 0.05$)

[a] Means of 30 (for main effects) or 10 (for interactions) replicates, 20 second-stage juveniles (J2) per replicate

[b] There was a treatment × cover crop interaction ($P \leq 0.05$) in the number of endospores per juvenile

[c] Plant performance rating scale: 0 = good stand of plants with a closed canopy and 5 = poor stand of plants with sparse canopy

[d] Galling index: 0 = no galls, 1 = 1–10, 2 = 11–20, 3 = 21–55, 4 = 56–80, and 5 = 81–100% of roots or pods galled

[e] Means of less than 20 J2 per replicate.

TABLE 2-2. Effect of *Meloidogyne arenaria* (RKN) alone and in combination with *Pasteuria penetrans* (RKN + Pp) and of a rye, wheat, or bare fallow winter cover crop on yield and performance of peanut in the 6th year (1992) of a 6.5 year multiplot experiment.

		12 per 100 cm³ of soil		% J2 with attached endospores		Endospores per juvenile		Plant performance rating		Galling index[b]	
		Spring	Fall	Spring	Fall	Spring	Fall	Yield (g/plot)	Shoots[c]	Roots	Pods
Main effects											
Treatment	Control	0.1 b	103 b	0 b	0 b	0 b	0 c	408 a	3.7 a	0.2 b	0.2 b
	RKN	110 a	1 239 a	19 a	36 a	5 a	8 b	190 b	2.1 b	3.2 a	3.2 a
	RKN + Pp	2 b	1 c	36 a	27 a	9 a	22 a	400 a	4.0 a	0 b	0 b
Cover crop	Rye	28 b	385 b	18	24	4	13 a	340	3.3	1.0	1.0
	Wheat	32 b	476 a	15	23	3	4 b	336	3.4	1.2	1.3
	Fallow	53 a	483 a	22	50	8	12 a	322	3.0	1.2	1.2
Interactions											
Rye	Control	0	0	0	0	0	0 c	404	3.8	0	0
	RKN	83	1 154	23	32	6	5 b	210	2.2	3.1	2.9
	RKN + Pp	1	2	30[e]	41[e]	5[e]	34[e] a	405	4.0	0	0
Wheat	Control	0.2	220	0[e]	0	0[e]	0 b	400	3.7	0.5	0.5
	RKN	93	1 206	19	48	4	8 a	198	2.4	3.2	3.4
	RKN + Pp	1.9	0.5	28[e]	20[e]	8[e]	4[e] a	408	4.1	0	0
Fallow	Control	0	90	0	0	0	0 c	419	3.7	0.2	0.2
	RKN	156	1 357	15	23	7	10 b	161	1.7	3.4	3.4
	RKN + Pp	1.6	0.8	50[e]	20[e]	16[e]	27[e] a	386	3.7	0	0

[a] Means followed by no letter or the same letter within a column of three observations for main effect or 10 observations for interactions were not different according to Duncan's multiple range test ($P \leq 0.05$).

[b] Means of 30 replicates (for main effects) or 10 replicates (for interactions). 20 second-stage juveniles (J2) per replicate.

[c] Plant performance rating scale: 0 = good stand of plants with a closed canopy and 5 = poor stand of plants with sparse canopy.

[d] Galling index. 0 = no galls, 1 = 1–10, 2 = 11–20, 3 = 21–55, 4 = 56–80, and 5 = 81–100% of roots or pods galled.

[e] Means of less than 20 J2 per replicate.

TABLE 2 3 Effect of *Meloidogyne arenaria* (RKN) alone and in combination with *Pasteuria penetrans* (RKN + Pp) and of a rye, wheat or bare fallow winter cover crop on yield and performance of peanut in the 7th year (1993) of a 6 5 year m cropiat experiment

		J2 per 100 cm³ soil		% J2 with attached endospores		Endospores per juvenile		Plant performance Yield g/plot	Shoots[a]	Galling index[b] Roots	Pods
		Main effects									
		Spring	Fall	Spring	Fall	Spring	Fall	Fall	Fall	Fall	Fall
Treatment	Control	0 b	0 b	0 b	8 c	0 b	3 b	218	2.6	0 b	0 b
	RKN	79 a	339 a	29 a	91 a	4 a	34 a	204	2.4	1 3 a	0.6 a
	RKN + Pp	0 b	3 b	10 a	33 b	2 a	16 a	217	2.9	0 b	0 b
Cover crop	Rye	17 b	125 ab	12	42	1 2 b	16 b	210	2.7	0.4	0 2
	Wheat	26 b	93 b	14	50	3 a	25 a	229	2.7	0.5	0 1
	Fallow	36 a	131 a	13	41	1 2 b	12 b	200	2.5	0.5	0 2
		Interactions									
Rye	Control	0 b	0 b	0	13[c]	0	0.2[c]b	212	2.4	0	0
	RKN	52 a	352 a	26	92	3	38 a	228	2.9	1 1	0.6
	RKN + Pp	0 b	2 b	10[c]	20[c]	0.8[c]	9[c]ab	190	2.7	0	0
Wheat	Control	0 b	0 b	0	10[c]	0	5[c]b	222	2.7	0	0
	RKN	78 a	278 a	31	100	5	36 a	220	2.2	1 4	0.4
	RKN + Pp	0 b	1 b	10[c]	40[c]	4[c]	29[c]a	244	3 2	0	0
Fallow	Control	0 b	0 c	0[c]	0	0	0 c	220	2.7	0	0
	RKN	107 a	387 a	30	82	3	27 a	165	2.0	1 5	0.7
	RKN + Pp	0 b	6 b	10[c]	40[c]	0.3[c]	10[c]b	216	2.8	0	0

Means followed by no letter or the same letter within a column of three observations for main effect or 10 observations for interactions were not different according to Duncan's multiple range test ($P \leq 0.05$)

Means of 30 replicates (for main effects) or 10 replicates (for interactions) 20 second-stage juveniles (J2) per replicate

There were treatment × cover crop interactions ($P \leq 0.05$) in the number of ring nematodes and the number of J2 per 100 cm³ soil

[a] Plant performance rating scale. 0 = good stand of plants with a closed canopy and 5 = poor stand of plants with sparse canopy

[b] Galling index. 0 = no galls 1 = 1 10 2 = 11 20, 3 = 21 55 4 = 56-80, and 5 = 81 100% of roots or pods galled

[c] Means of less than 20 J2 per replicate

inoculated with *P. penetrans* was nearly undetectable in soil samples from 1991 to 1993. The number of *P. penetrans* endospores adhering to J2 increased to a high of 17.2 by 1989 and remained relatively consistent during the remaining 4-year period (Oostendorp et al. 1991a; Fig. 2-1; Tables 2-1 to 2-3). Through this time, 10-66% of the J2 were encumbered with endospores. However, when replicates without J2 were excluded from analysis for RKN + Pp plots, the incidence of endospores per juvenile and attachment to J2 were much higher than those for 20 J2 (Table 2-4). In the fall of 1990, the endospore density of *P. penetrans* in RKN + Pp plots had amplified to yield an average of 19.5 endospores/juvenile (data not shown). Between 1991 and 1993, the endospore density remained at a level at which 95% to 100% of the recoverable J2 were encumbered with mean numbers of 3 to 72 endospores (Table 2-4).

In the soil of RKN plots, the number of J2 remained high throughout the experiment (Oostendorp et al. 1991a; Fig. 2-1; Tables 2-1 to 2-3). In the third year (1989), 2 of the 30 replicates of the RKN plots were contaminated with the bacterial parasite (Oostendorp et al. 1991a), and *P. penetrans* endospores were found to have contaminated 16, 29, and all 30 RKN plots in the falls of 1991, 1992, and 1993, respectively (Figs. 2-2 to 2-4; Tables 2-1 to 2-3). In fall of 1993, endospore population densities of RKN plots had increased to averaged 91% attachment rate with 34 endospores/juvenile (Table 2-3).

Numbers of nematodes and endospore attachment levels generally did not vary with cover crops ($P \leq 0.05$) (Fig. 2-5; Tables 2-1 to 2-3). However, in the spring of 1989, the nematode population densities in RKN plots under vetch slightly exceeded those of other plots (Fig. 2-5A). Second-stage juvenile numbers in vetch and bare fallowed plots exceeded those in the rye plots in the spring of 1990. However, in the spring of 1992 and spring of 1993, lower numbers of J2 were recovered from plots under rye and wheat than

TABLE 2-4 The percentage of second stage juveniles (J2) of *Meloidogyne arenaria* infected with *Pasteuria penetrans* and the average number of endospores per juvenile in peanut microplots infested with *M. arenaria* and *P. penetrans* in the spring of 1987 and rotated with rye, vetch or wheat, and bare fallow as winter cropping sequence

Cover crop	% J2 with endospores attached	Endospores/juvenile	% J2 with endospores attached	Endospores/juvenile
			Fall 1991	
Rye			100 (5)	10 (5)
Vetch			(0)	(0)
Fallow			100 (18)	40 2 (18)
	Spring 1992		Fall 1992	
Rye	100 (12)	17.8 (12)	66 7 (17)	32 4 (17)
Wheat	100 (19)	35 3 (19)	66 7 (5)	8 5 (5)
Fallow	95 (16)	21 6 (16)	100 (8)	44 1 (8)
	Spring 1993		Fall 1993	
Rye	100 (1)	6 (1)	100 (16)	43 (16)
Wheat	100 (1)	42 (1)	100 (9)	72 (9)
Fallow	100 (1)	3 (1)	80 (62)	20 (62)

Means of varying number of replicates

Numbers in parenthesis are the number of nematodes observed

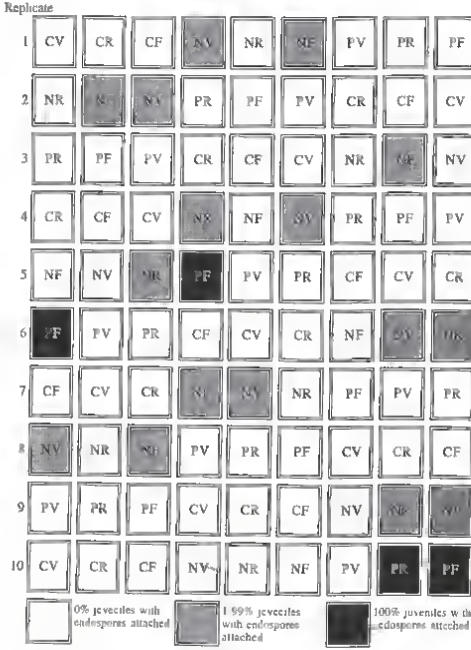
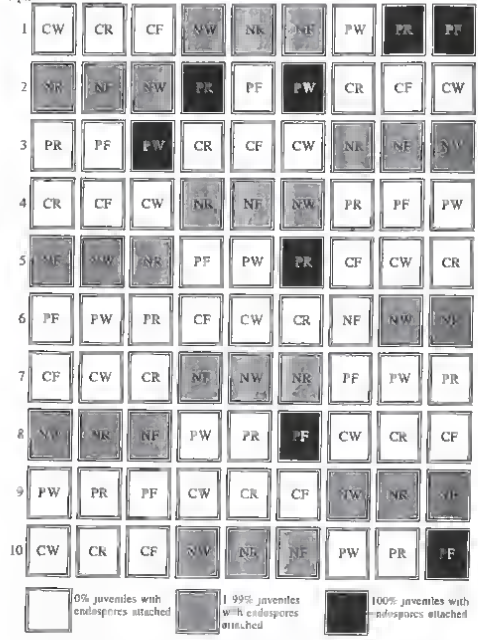

Replicate
1 CV CR CF NV NR NF PV PR PF
2 NR NF NV PR PF PV CR CF CV
3 PR PF PV CR CF CV NR NF NV
4 CR CF CV NR NF NV PR PF PV
5 NF NV NR PF PV PR CF CV CR
6 PF PV PR CF CV CR NF NV NR
7 CF CV CR NF NV NR PF PV PR
8 NV NR NF PV PR PF CV CR CF
9 PV PR PF CV CR CF NV NF NV
10 CV CR CF NV NR NF PV PR PF
0% juveciles with endospores attached
1-99% juveciles with endospores attached
100% juveniles with endospores attached

Fig 2.2 Microplot treatment plan indicating the presence of *Pasteuria penetrans* endospores as determined by soil sample analysis at the fall of 1991. No second stage juveniles were extracted from non-shaded plots. The first letter indicates the pre experimental treatment (C = plots free of nematodes and *P. penetrans* N = plots inoculated with *Meloidogyne arenaria* and P = plots inoculated with *M. arenaria* plus *P. penetrans*) and the second letter indicates the winter cover crops (V = vetch R = rye, and F = fallow)


Replicate
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2 NR NF NW PR PF PW CR CF CW
3 PR PF PW CR CF CW NR NF NW
4 CR CF CW NR NF NW PR PF PW
5 NF NW NR PF PW PR CF CW CR
6 PF PW PR CF CW CR NF NW NF
7 CF CW CR NF NW NR PF PW PR
8 NW NR NF PW PR PF CW CR CF
9 PW PR PF CW CR CF NW NR NF
10 CW CR CF NW NR NF PW PR PF
0% juvenles with endospores attached
1-99% juvenles with endospores attached
100% juvenles with endospores attached

Fig 2.3 Microplot treatment plan indicating the presence of *Pasteuria penetrans* endospores as determined by soil sample analysis in the fall of 1992. No second-stage juveniles (J2) were extracted from non shaded plots. The first letter indicates the pre experimental treatment (C = plots free of nematodes and *P. penetrans*; N = plots inoculated with *Meloidogyne arenaria* and P = plots inoculated with *M. arenaria* plus *P. penetrans*) and the second letter indicates the winter cover crops (W = wheat, R = rye and F = fallow).

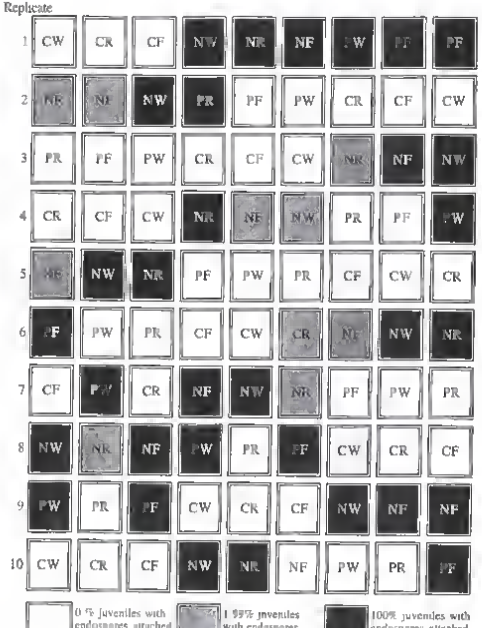
Replicate
1 CW CR CF NW NR NF PW PR PF
2 NR NF NW PR PF PW CR CF CW
3 PR PF PW CR CF CW NR NF NW
4 CR CF CW NR NF NW PR PF PW
5 NF NW NR PF PW PR CF CW CR
6 PF PW PR CF CW CR NF NW NR
7 CF PF CR NF NW NR PF PW PR
8 NW NR NF PW PR PF CW CR CF
9 PW PR PF CW CR CF NW NF NF
10 CW CR CF NW NR NF PW PR PF
0 % juveniles with endospores attached
1-99% juveniles with endospores attached
100% juveniles with endospores attached

Fig 2-4 Microplot treatment plan indicating the presence of *Pasteuria penetrans* endospores as determined by soil sample analysis in the fall of 1993. No second stage juveniles (J2) were extracted from non-shaded plots. The first letter indicates the pre-experimental treatment (C = plots free of nematodes and *P. penetrans*; N = plots inoculated with *Meloidogyne arenaria* and P = plots inoculated with *M. arenaria* plus *P. penetrans*) and the second letter indicates the winter cover crops (W = wheat, R = rye and F = fallow).

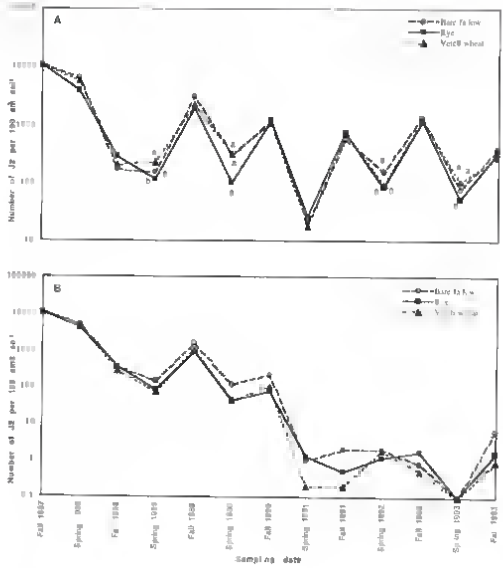

Fig 2.5 Number of second stage juveniles (J2) of *Meloidogyne arenaria* race 1 in 100 cm³ of soil extracted from soil in microplots with A) *M. arenaria* and B) *M. arenaria* plus *P. penetrans*. Plots were planted to peanut in summer and bare fallowed or planted to rye or vetch (1987 1990) and wheat (1991 1992) in winter. Means of the cropping sequences at individual sampling dates labeled by no or the same letter are not different according to Duncan's multiple-range test ($P \leq 0.05$)

rye and wheat than from those under bare fallow ($P \leq 0.05$). In the fall of 1991, in RKN plots the estimated endospore population density in vetch wheat plots was highest and together with rye plots, exceeded the endospore counts of fallow plots in the fall of 1993 ($P \leq 0.05$) (Fig. 2-6 A). From the spring of 1989 till the spring of 1990, the attachment level and the number of endospores per juvenile in RKN + Pp plots under vetch were higher than under bare fallow ($P \leq 0.05$) (Fig. 2-6 B). Thereafter, the endospore counts in vetch wheat were frequently lower than in bare fallow plots. In the fall of 1993, the vetch wheat plots yielded the highest estimated endospore population density. In the fall of 1990 and of 1992, the endospore counts in rye plots exceeded those of bare fallow plots and vetch wheat plots, respectively.

According to main effect means in the spring and the fall of 1993, the attachment rates of 3 and 25 endospores per juvenile across all nematode treatments in wheat plots were higher ($P \leq 0.05$) than the levels observed in rye or fallowed plots (Table 2-3). The highest number of 107 J2/100 cm^3 of soil in fallowed RKN plots observed in the spring of 1993 was numerically greatest, but yet consistent with the main effect of highest juvenile levels in RKN plots.

Following the initial increase in peanut yield in RKN + Pp plots after 3 years, the yields remained high and not different from those in control plots for the remaining 4 years ($P \leq 0.05$) (Fig. 2-7, Tables 2-1 to 2-3). In 1993, there were no differences among yields in any treatments, which is attributed to *P. penetrans* infesting RKN plots (Fig. 2-6 A, Table 2-3). *Meloidogyne arenaria* was low or undetectable in control plots throughout the experiment. Throughout the last 4 years, the galling indices for roots and pods in RKN plots were higher than gall ratings of RKN + Pp and control plots ($P \leq 0.05$) (Tables 2-1 to 2-3).

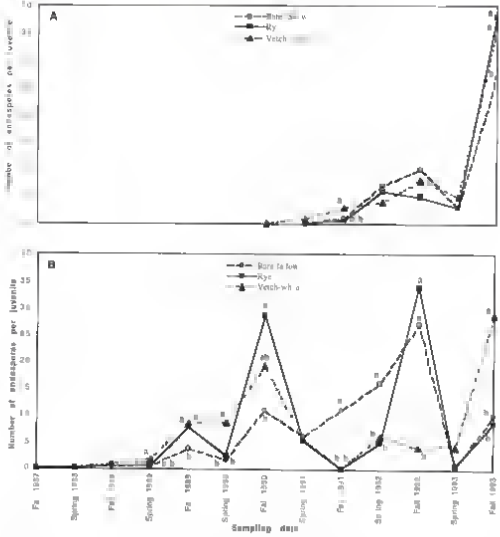

Fig 2-6 Number of attached endospores per juvenile extracted from soil in microplots with A) *M arenaria* and B) *M arenaria* plus *P penetrans* Plots were planted to peanut in summer and bare fallowed or planted to rye or vetch (1987 1990) and wheat (1991 1992) in winter Means of the cropping sequences at individual sampling dates labeled by no or the same letter are not different according to Duncan s multiple range test ($P \leq 0.05$) Means of 10 replicates with 20 or fewer J2 observed per replication

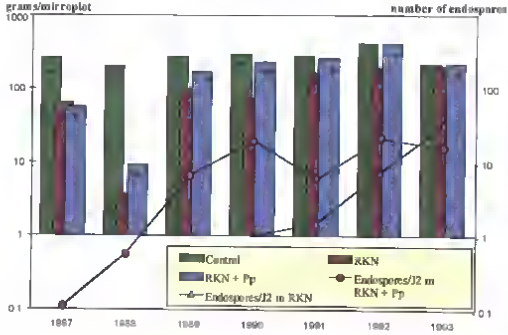

Fig. 2-7 Log presentation of peanut yield (grams per microplot) from plots treated with *Meloidogyne arenaria* race 1 (RKN) and *M. arenaria* plus *Pasteuria penetrans* (RKN + Pp) and untreated plots and the number of endospores per second-stage juvenile (J2) in RKN and RKN + Pp plots. Data from 1987 to 1989 were from Oostendorp et al. 1991a. Means of 10 replicates, with 20 or fewer juveniles observed per replication to determine the number of endospores per J2. Means with the same letter are not significantly different according to Duncan's multiple-range test ($P < 0.05$).

Bar graphs—peanut yields in grams, line graphs—number endospores per juvenile

Throughout the last 3 years of the experiment (1991 to 1993) the population density development of *Criconemella* spp was favored in plots that were fallowed through the winter ($P \leq 0.05$) (Appendix). An interaction between treatment and cover crop showed a higher number of ring nematodes in fallowed RKN plots than in fallowed RKN + Pp plots. In the fall of 1991 the highest number of ring nematodes in the soil coincided with the highest number of endospores of *P. penetrans* attached per juvenile of *Meloidogyne arenaria*.

Discussion

Populations of *P. penetrans*, as estimated by endospore attachment to juveniles, increased over 6.5 years from relatively low levels of endospores added initially or introduced by contamination during the course of the experiment to levels after 3 years that were suppressive to the nematode population. This confirms the work by Oostendorp et al. (1991a) and Chen et al. (1997c) whereby *P. penetrans* endospores increased to levels that suppressed root knot nematodes when introduced at low numbers (approximately 1,000 spores/g of soil). Within four cropping seasons, nematode attachment rates in plots with endospores added increased to nearly 100% with an average of about 20 endospores per juvenile, and peanut yields reached levels equivalent to those in the nematode-free control.

The population density of *M. arenaria* in RKN + Pp plots remained nearly undetectable throughout the last 3 years of this 6.5-year experiment. It was difficult to obtain an estimate of endospores per juvenile over the course of the experiment because the number of J2 in many RKN + Pp plots dropped below 20, which was the number used for estimating the endospore density. This was illustrated in the fall of 1991 when a decrease

in the number of endospores per juvenile and a decrease in the percentage of J2 with attached endospores were observed in rye and vetch plots. Individual observations revealed a further increase in the endospore population density under either cropping sequence. In 1992 and 1993 the attachment rates of juveniles with *P. penetrans* were around 100% and the number of endospores per juvenile frequently exceeded 25. At this rate root penetration has been shown to be prevented (Davies et al., 1988; Stirling, 1984; Stirling et al., 1990). Nematode suppression for three consecutive years did not affect the ability of *P. penetrans* endospores to attach to their nematode hosts. However, the experimental design did not allow for the determination of the number of J2 that may have inadvertently entered the microplots each season. Although it has been reported that the suppressiveness of root-knot nematode by *P. penetrans* is dependant on the density of endospores in the soil (Stirling, 1991), it appears in the present study that *P. penetrans* increased to a suppressive level after 3 years and remained sufficiently high to prevent further increases in the RKN densities. *Pasteuria penetrans* remained at a highly suppressive level for 3.5 years following the initial increase to suppressive levels in 3 years. The nematode population density required to maintain effective densities of *P. penetrans* to continue nematode suppression remains unknown.

In 1991 and 1992, the residual nematode population in RKN plots reduced peanut yields and affected the plant performance. However, in 1993, plant shoots and yields appeared to have been affected by leaf chlorosis and reduced fruiting, apparently as a result of the symptomatic manganese deficiency (Dickson, personal communication; Porter et al., 1984), rather than by nematode infection. There was only slight galling of roots, pods, and pegs by root knot nematodes in the RKN plots in 1993, which indicates that nematode reproduction was becoming suppressed, and that the *P. penetrans* population density in the soil was continuing to increase to suppressive levels.

With no *P. penetrans* to interfere, the nematode population density peaked when peanut was followed by a susceptible crop. Those conditions favored the contamination by and the build up of endospores of *P. penetrans*. This observation agrees with those of other scientists (Chen et al., 1994; Maduln et al., 1994; Oostendorp et al., 1991a) in which the continuous availability of a host plant favored the amplification of the bacterial parasite in its nematode host. However, since the nematode population density in the presence of *P. penetrans* did not differ among the cropping sequences, it is suggested that nematodes in vetch plots were already partially suppressed in years 2 and 3 of the experiment. Because of the density dependent nature of *P. penetrans* (Bird and Brisbane, 1968; Chen et al., 1996b; Dickson et al., 1994; Minton and Sayre, 1989; Stirling, 1984), the endospore population development is expected to be influenced by the population density of its nematode host (Stirling, 1991).

The winter cover crop had a consistent effect on the population density of *Criconemella* spp. Following the peanut microplots, mereased the number of ring nematodes more than growing rye and wheat or vetch for winter cover. *Criconemella* sp. was reported as a host for *Pasteuria* spp. (Sturhan, 1985; 1988). However, endospores of isolates of *Pasteuria* spp. from *Criconemella* spp. were smaller and are believed to be a different species (Hewlett, personal communication); hence, they do not likely contribute to the suppression of *M. arenaria* in the peanut microplots.

For a period of 3 years (1991 to 1993) *P. penetrans* protected peanut from root-knot damage. The presence of a large population of *M. arenaria* in the RKN plots favored a continuous endospore population increase until the nematode population was detrimentally affected after a period of 4 years (1990 to 1993), which resulted from inadvertent contamination by *P. penetrans* endospores.

The build-up of the endospore density in the soil can be enhanced by supplying the nematode population with a good host plant throughout the year Thus although there is still no large scale production of *P. penetrans* inoculum in sight, the use of this nematode antagonist in integrated nematode management programs might be feasible There is a need for further experiments to determine whether the nematode suppression can be achieved in a shorter time span, and whether it can be maintained beyond a period of 3 years

CHAPTER 3
USE OF MICROWAVE HEATING IN EVALUATION OF A *MELOIDOGYNE ARENARIA* SUPPRESSIVE SOIL CONTAINING *PASTEURIA PENETRANS* AND ITS APPLICATION IN A SUPPRESSIVE-SOIL TEST

Introduction

Pathogen suppressive soils may be defined as soils in which the pathogen does not establish or persist, establishes but causes little or no damage, or establishes and causes disease (conducive soil) but thereafter the disease is reduced even though the pathogen persists in the soil (Baker and Cook 1982). Suppressiveness can develop as a result of the buildup of antagonists in response to a high pathogen population (Baker and Cook 1982), especially in situations where susceptible crops are grown in succession.

The endospore-forming obligate bacterial parasite of root-knot nematodes *Pasteuria penetrans* (Thorne) Sayre & Starr is widely distributed in agricultural soils throughout the world (Chapter 1) and has been shown to be effective in controlling root-knot nematodes *Meloidogyne* spp. in field or microplot studies (Brown and Smart, 1985; Chen et al., 1994, 1996b, 1997c; Dickson et al. 1994; Minton and Sayre, 1989; Oostendorp et al. 1991a). In most cases, the suppression of *Meloidogyne* spp. by *P. penetrans* occurred after long-term monoculture of a susceptible host (Bird and Brisbane 1988; Chen et al. 1994; Mankau, 1980a; Minton and Sayre, 1989; Weibelzahl-Fulton et al. 1996).

Many physical and chemical means have been developed to eliminate or reduce populations of soilborne plant pathogens for phytopathological studies (Mulder 1979). Nematode suppressiveness of soil has been evaluated previously using soil applications

of fungicides, such as captafol (Crump, 1987), mancozeb and iprodione (Mertens and Starling 1993), benomyl or tachigaren (Qadri and Saleh 1990) and 40% formaldehyde (Dickson et al., 1994; Kerry et al., 1982 Qadri and Saleh 1990) Suppression of fungal populations by fungicides is not consistent, thus the interpretation of results can be problematic. Microwave heating of soil for selective periods of time can be useful to kill or reduce populations of soilborne fungal propagules yet have little effect on populations of prokaryotic organisms (Chen et al 1995, Ferriss 1984) The effect of the microwave radiation treatment on microorganisms increases with increasing temperature. The temperature increase depends on treatment time and on factors such as soil water clay, and organic matter content (Baker and Fuller 1969 Ferriss 1984)

The objectives of this study were to determine if the microwave radiation treatment has the potential to selectively eliminate fungal antagonists from soils containing *P penetrans* and to evaluate the use of the microwave treatment in testing three microplot soils for suppressiveness to *M arenaria*

Materials and Methods

Microwave Treatment

Treatment time. On 12 January 1993, approximately 12 liters of soil in loamy, siliceous, hyperthermic Grossarenic Paleudults with 90% sand 4% silt, 6% clay 1.8% organic matter 6.9% moisture and a density of 2.78 were collected 0-20 cm deep with a bucket auger (10-cm diameter) from microplots infested with *P penetrans* and *Meloidogyne arenaria* race 1 located at the University of Florida, Green Acres Agronomy Farm, Alachua County Soil was selectively collected from microplots that were bare

fallowed during the winter. The soil was passed through a 5 mm-aperture sieve, and mixed thoroughly. Six subsamples of 1 kg each were placed in open polypropylene bags leveled to a thickness of approximately 5 cm and placed centrally in the 18 liter cavity of a microwave oven (1,500 Watts 2,450 MHz) (Tappan Appliance, Mansfield, OH). The subsamples were either microwave treated for 3, 4, 5, 6, and 8 minutes or left untreated. The bags remained open through the heat treatment and were closed before removal from the oven. The soil was allowed to cool to room temperature.

Two hours later a dilution plating technique was used to assay for selected fungi (Johnson and Curl, 1972). One gram of soil was dispersed in 200 ml of sterilized water and stirred constantly. One milliliter of the soil solution was plated on potato dextrose agar containing 50 mg of chlortetracycline hydrochloride, 100 mg of streptomycin sulfate, and 1 ml of Tergitol NP10 per 1 liter solution (supplements were added to agar at a temperature of $\leq$ 50 °C) (Steiner and Watson, 1965). Five replicates were plated for each treatment. After 3 days of incubation at room temperature, the fungal colonies were counted.

A soil bioassay with *Meloidogyne arenaria* race 1 as the host nematode was used to determine the presence of *P. penetrans* (Oostendorp et al., 1991a). Forty grams of dry soil from each treatment were placed in each of five, 10-cm-diameter petri dishes. Then, 300 second stage juveniles (J2) were added to 1 ml of water to each dish. After 2 days of incubation at room temperature, the J2 were extracted by a centrifugal-flotation method (Jenkins, 1964), and the number of endospores per 20 randomly selected J2 per replicate was determined with the aid of an inverted microscope at ×400 magnification.

Soil moisture content. On 8 March 1993, 1.5 kg lots of the dried soil collected on 12 January 1993 were placed into plastic bags, and the soil moisture content was adjusted to 1%, 3%, 5%, or 7% (weight of water per dry soil weight) by adding tap water. After 2 hours, the soil was mixed thoroughly, divided into three subsamples, placed into open

polypropylene bags, and was either heated in the microwave oven for 3 or 4 minutes per kilogram of soil or left untreated. The numbers of fungal colonies per gram of soil and endospores per juvenile were determined as described above.

Suppressive-Soil Test

Soil treatments. On 23 April 1993 and 7 May 1993, a total of 120 liters of soil was collected from the same microplots as stated above. For the repeat test, soil was collected on 23 April and 5 June. For each experiment, a subtotal of 40 liters of soil was collected from microplots with *M. arenaria* inoculum alone (RKN), *M. arenaria* plus *P. penetrans* inoculum (RKN + Pp), and a control treatment without nematodes or bacteria (control) (for more information on the soil sources see Chapter 2). The soils were passed through a 5-mm-aperture sieve, mixed, and divided into four subsamples of 10 liters each. The subsamples were either autoclaved, microwaved, air dried, or left untreated.

For the autoclave treatment, sterilized 15-cm-diameter clay pots were filled with soil (ca. 1 liter/pot), covered with aluminum foil, and placed in an autoclave. After the first heat treatment for 1.5 hours at 55 kPa (Ferriss, 1984), the pots were removed from the autoclave. The treatment was repeated after pots and soil had cooled to room temperature (Mulder, 1979).

For the microwave heating treatment, 1 kg lots of soil were placed in even layers in open polypropylene bags and heated in a microwave oven for 3 minutes (Ferriss, 1984) at full power, which caused the soil temperature to rise to about 75 °C.

For the air-drying treatment, 40 liters of soil were collected from the microplots on 7 May and 5 June 1993. The soil was placed in 3- to 5-cm-thick layers on plastic trays and

stored in the greenhouse for 2 or 6 weeks before testing. The untreated soil was stored at room temperature, and used within 24 hours after collection.

Laboratory experiment. Two hours after microwaving soil and the last autoclaving treatment, and 2 weeks after the beginning of the air dried treatment, the number of fungal colonies per gram of soil and endospores per juvenile was determined simultaneously for all four soil treatments. Procedures were applied as described above.

Greenhouse experiments. Treatments were arranged in a 3 × 4 × 2 factorial design that included the following three soil sources: soil from microplots with *M arenaria* inoculum alone (RKN), *M arenaria* plus *P penetrans* inoculum (RKN + Pp) and a control treatment without nematodes or bacteria (control), four soil treatments (autoclaved, microwaved, air-dried, and untreated), and inoculum levels of 0 and 2 000 J2 of *M arenaria* race 1. The soil from each treatment was placed into sterilized 15-cm-diameter clay pots (ca. 1 liter/pot). Treatments were replicated four and five times in experiments 1 and 2, respectively. About 100 ml and 50 ml of water were added to the air-dried soil and to all other soil treatments respectively. Each pot was covered with aluminum foil and held for 3 days in a growth room at 28 to 32 °C and 14 hours of light, or in a greenhouse for experiments 1 and 2, respectively. *Meloidogyne arenaria* J2 were suspended in 10 ml of water and dispensed equally to each of five holes, 5-cm deep, in the soil of each pot. Five days later, one 7 week old peanut seedling *Arachis hypogaea* L. cv Florunner, for experiment 1, or one 5 week-old peanut seedling of the same cultivar for experiment 2 was transplanted into each pot. A commercial *Rhizobium* sp. was added to each pot (McSorley et al., 1992). Every 10 to 14 days, insects and diseases were controlled using esfenvalerate, chlorpyrifos, insecticidal soap, chlorothalonil, or liquid sulfur.

Experiments 1 and 2 were run for 53 and 55 days respectively. Plant shoots were cut off at ground level and discarded. The root systems were washed free of soil, excess water was removed with paper towels, and roots were weighed and then stored in plastic bags at 4 °C. The following day, roots were stained with Phloxine B (Dickson and Strubel 1965), and the numbers of root galls and egg masses were counted. Eggs were extracted with 1.05% sodium hypochlorite (Hussey 1971) and their number was counted. Twenty globular mature females were picked randomly from each root system, crushed on a slide and observed for infection by *P. penetrans* with the aid of a compound microscope at ×1000 magnification.

Nematode Origin

The isolate of *M. arenaria* race 1 used originated from peanut grown on the University of Florida, Green Acres Agronomy Research Farm, Alachua County. The species and race determinations were confirmed by examination of the perineal pattern, length of J2, and a differential host test (Hartman and Sasser, 1985). The nematode population was cultured in a greenhouse on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) from a single egg mass. Eggs of *M. arenaria* were extracted from infected tomato roots (Hussey and Barker 1973), hatched by the Baermann method (Rodríguez-Kábana and Pope 1981) and used as 1- to 4-day-old J2.

Statistical Analysis

The number of endospores per juvenile was transformed with $\log_{10}(x + 1)$ before analysis. The percentages of female infection were transformed to arcsin ($\sqrt{x}$) before analysis. All data were subjected to factorial analysis of variance (ANOVA). Means were

separated and compared by Duncan's multiple range test. Regressions were performed to determine the relationship between the microwave treatment time and the survival of *P. penetrans* and fungal populations.

Results

Microwave Treatment

Treatment time. Population densities of *P. penetrans* endospores and fungi decreased with the increasing treatment time ($Y = 1.95x^2 - 30.31x + 115.67$) (Fig. 3-1). Following treatment for 3 minutes, the attachment of endospores was reduced by almost 50% (average of 59 endospores/juvenile). When the treatment time was extended to 4 minutes/kg of soil and 5 minutes/kg of soil, the attachment was reduced to 20 endospores per juvenile and nearly eliminated, respectively. The number of fungal colonies was greatly reduced by all treatment times, as compared to the untreated soil ($Y = 1.04x^2 - 12.21x + 33.69$). *Fusarium* sp., *Paecilomyces lilacinus*, *Penicillium* sp., *Pythium* sp., *Trichoderma* sp., and a variety of unidentified fungi were isolated.

Soil moisture content. The attachment of *P. penetrans* endospores to J2 increased slightly with increasing soil moisture content ($Y = -1.875x^2 + 27.4x + 10.28$) (Fig. 3-2). Numbers of endospores attached per juvenile decreased in soils of all tested moisture levels treated for 3 and 4 minutes in the microwave ($P \leq 0.05$) (Fig. 3-3). A further decrease in attachment was observed with increased treatment time in soils containing 3, 5, and 7% moisture. These observations validate the numbers observed in the treatment-time study reported above (Fig. 3-2). However, the endospore counts of this study were much lower

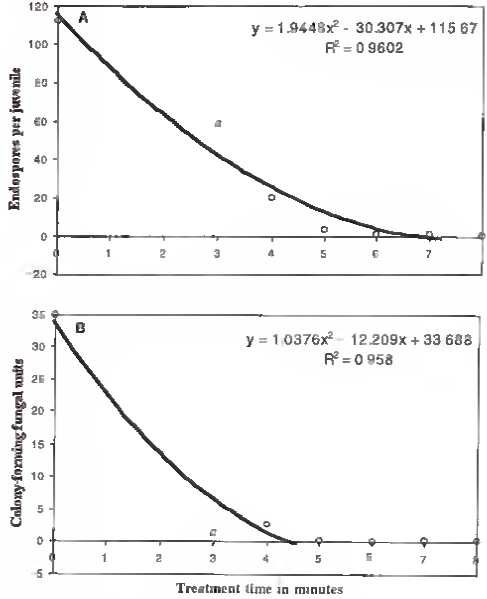

Fig 3-1 Effect of microwave radiation treatment of 1 kg of soil containing *Pasteuria penetrans* on A) the attachment of *P. penetrans* endospores to *Meloidogyne arenaria* race 1 and B) the survival of soilborn fungi as determined by the number of colony-forming units. Values are means of five replications. 20 randomly selected second stage juveniles were observed.

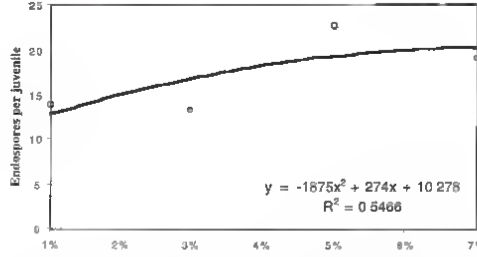

Fig. 3-2. Relationship between the soil moisture content and the number of endospores of *Pasteuria penetrans* attached per second stage juvenile (J2) of *Meloidogyne arenaria* race 1 after 48 hours exposure at room temperature. Values are means of two replications. 20 randomly selected J2 were observed.

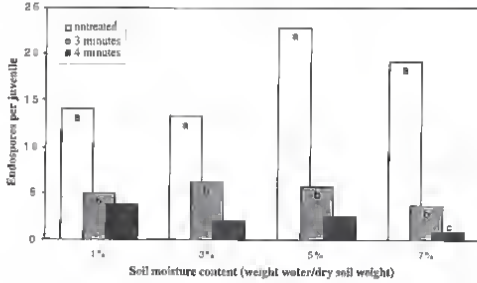

Fig. 3-3. Effect of soil moisture content and microwave treatment time on the attachment of *Pasteuria penetrans* endospores to *Meloidogyne arenaria* race 1. Values are means of two replications. 20 randomly selected second-stage juveniles were observed. Means within each moisture treatment followed by the same letter do not differ at $P \leq 0.05$ according to Duncan's multiple-range test.

than those of the treatment time study

Fungal populations were detected at all moisture levels of the untreated soil only Population densities increased with increasing moisture content of the soil ($Y = 21x + 1$, $R^2 = 0.99$) (data not shown) and were 1.2×10^3, 1.7×10^4, 2×10^4, and 2.5×10^5 colony forming units (cfu)/g of soil containing 1% 3% 5% and 7% moisture respectively

Suppressive-Soil Test

Laboratory experiment. Survival of fungi and *P. penetrans* in the microplot soil varied with soil sources and soil treatments ($P \leq 0.05$) (Table 3-1) Regardless of soil source populations of fungi were lower in autoclaved or microwaved soil than in air dried and untreated soil ($P \leq 0.05$) The fungal populations in soil from RKN plots generally exceeded those in soil from RKN + Pp plots and control soils in untreated or in air dried soils No *P. penetrans* endospores were detected in control soil or in any autoclaved soils. The number of endospores attached per juvenile did not differ among the microwaved, air dried, and untreated soils.

Greenhouse experiments. The effects of the soil sources treatments, and inoculation levels were similar in both experiments for all data, except the number of eggs as affected by the soil treatments and the female infection rate (Table 3-2). In experiment 2 the number of eggs did not vary with soil treatments and the single interaction affecting the female infection rate was the soil source × soil treatment interaction

Reproduction of *M. arenaria* on peanut as expressed by the number of root galls, egg masses, and eggs, was consistently higher in autoclaved soil than in microwaved, air dried and untreated soil infested with *M. arenaria* alone (Table 3-3) or *M. arenaria* plus *P. penetrans* (Table 3-4) after inoculation with 2 000 J2 of *M. arenaria* In soil infested

TABLE 3-1 Colony forming units (cfu) of soil fungi and attachment of *Pasteuria penetrans* endospores to second-stage juveniles (J2) of *Meloidogyne arenaria* in untreated soil and soil autoclaved twice for 1 5 hours at 55 kPa, microwaved for 3 minutes/kg of soil, or air dried for 2 weeks in the greenhouse Soils contained 6 9% moisture and were collected from peanut microplots infected with *M arenaria* inoculum alone (RKN) *M arenaria* plus *P penetrans* inoculum (RKN + Pp)- and a cautrol treatment without added nematodes or bacteria (control)

Soil source	Soil treatment	Cfu of fungi per gram of soil	Mean number of *P penetrans* endospores per juvenile
Control	Autoclaved	80 b A	0 a
	Microwaved	80 b A	0 a
	Air dried	1 1 x 10^6 a B	0 a
	Untreated	1 6 x 10^6 a B	0 a
RKN	Autoclaved	0 b A	0 b
	Microwaved	40 b A	1 1 ab
	Air-dried	3 4 x 10^4 a A	8 7 a
	Untreated	4 5 x 10^4 a A	9.5 a
RKN + Pp	Autoclaved	80 b A	0 b
	Microwaved	0 b A	9 5 a
	Air-dried	1.5 x 10^5 a B	12 4 a
	Untreated	1 5 x 10^4 a B	9 8 a

Values are means of five replicates for the cfu counts and two replicates for the endospore counts 20 randomly selected J2 were observed per replicate

The data were transformed with $\log_{10}(x+1)$ before being subjected to ANOVA Presented data are untransformed

Means within columns for each soil source or for each treatment followed by the same lower case letter or upper case letter,respectively, do not differ at $P \leq 0$ 05 according to Duncan s multiple range test

TABLE 3.2 ANOVA table for the effect of soil source (microplots infected with *Meloidogyne arenaria* race 1 alone, with *M. arenaria* race 1 plus *Pasteuria penetrans* and without either organism), soil treatments (autoclaved microwaved air-dried and untreated) and *M. arenaria* race 1 inoculum levels (0 or 2 900 second-stage juveniles) on nematode reproduction and fresh root weights of peanut cv. Florunner

Source of variation	Number of galls	Number of egg masses	Number of eggs	Females infected	Fresh root weight
		Experiment 1			
Soil source (S)	***	***	***	***	NS
Soil treatment (T)	***	***	***	**	NS
Inoculation level (I)	***	***	***	*	NS
S x T	***	***	***	**	NS
S x I	***	***	***	**	NS
I x T	***	***	***	**	NS
I x T x S	***	***	***	*	NS
		Experiment 2			
Soil source (S)	***	***	**	***	NS
Soil treatment (T)	***	***	NS	*	*
Inoculation level (I)	***	***	***	*	NS
S x T	***	**	***	*	NS
S x I	***	**	***	NS	NS
I x T	***	***	***	NS	NS
I x T x S	***	***	***	NS	NS

*, **, *** represents $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively
NS = nonsignificant at $P \leq 0.05$

TABLE 3 3. Effect of autoclaving, microwaving and air-drying of soil infested with *Meloidogyne arenaria* race 1 alone on the nematode reproduction percentage of females infected by *Pasteuria penetrans* and fresh root weights of peanut cv Florunner following inoculation with 0 or 2 000 second-stage juveniles (J2) [1]

Soil treatment	Nematode reproduction: Number of root galls per root system, Inoculation level		Number of egg masses per root system, Inoculation level		Number of eggs per root system, Inoculation level		% females infected (mean of 20 observations), Inoculation level		Fresh root weight (g), Inoculation level	
	+ [b]		+		+		+		+	
Experiment 1										
Autoclaved	605 a	0 b	522 a	0 b	98 750 a	0 b	0 b	[d]	4 8 a	4 5 a
Microwaved	18 b	0 b	5 b	0 b	1 150 b	0 b	10 [c] a		3 9 b	4 1 a
Air dried	0 c	0 b	0 c	0 b	40 c	0 b			3 3 b	4 1 a
Untreated	36 b	10 a	4 b	6 a	560 b	290 a	17 [c] a	77 [c] a	3 4 b	4 1 a
Experiment 2										
Autoclaved	491 a	0 b	380 a	0 b	116,960 a	0 b	0 b		4 1 a	2,5 b
Microwaved	0 b	0 b	0 b	0 b	40 b	0 b			4 7 a	4 2 a
Air dried	0,8 b	0 b	0 b	0 b	20 b	0 b	30 [c] a		3,5 a	3 9 b
Untreated	1 b	5 a	0,8 b	1 a	220 b	200 a	17 [c] a	69 [c] a	3 5 a	3 2 b

Values are means of four replicates in experiment 1 and five replicates in experiment 2. Means within columns in each experiment followed by the same letter do not differ at $P \leq 0.05$ according to Duncan's multiple range test

[a] The soil in the 6.5 year microplot experiment that was initially infested with *M. arenaria* alone became infested with *P. penetrans* endospores in the 4th year

[b] + Inoculated with 2 000 J2 of *M. arenaria* race 1 No nematodes added

[c] Less than 20 observations in at least one replicate

[d] – No observation

TABLE 3-4 Effect of autoclaving, microwaving, and air drying of soil infested with *Meloidogyne arenaria* race 1 and *Pasteuria penetrans* on the nematode reproduction, percentage of females infected by *P. penetrans*, and fresh root weights of peanut cv. Florunner following inoculation with 0 or 2 000 second-stage juveniles (J2) of *M. arenaria* race 1

Soil treatment	Nematode reproduction: Number of root galls per root system, Inoculation level		Number of egg masses per root system, Inoculation level		Number of eggs per root system, Inoculation level		% females infected (mean of 20 observations), Inoculation level		Fresh root weight (g), Inoculation level	
	+ [a]		+		+		+		+	
Experiment 1										
Autoclaved	702 a	0 a	608 a	0 a	137 370 a	0 a	0 b	– [c]	4 6 a	4 5 a
Microwaved	10 b	0 a	0 b	0 a	130 b	0 a	100 a		4 5 a	4 0 a
Air dried	8 b	0 a	0 b	0 a	60 b	0 a	100 a		3 3 b	4 2 a
Untreated	3 b	0 a	0 b	0 a	10 b	0 a	100 a		3 5 b	3 2 b
Experiment 2										
Autoclaved	300 a	0 a	256 a	0 a	72,788 a	0 a	0 b		3 5 a	4 5 a
Microwaved	6 b	0 a	3 b	0 a	260 b	0 a	77 [b] a		3 3 a	3,3 b
Air dried	0 b	0 a	0 b	0 a	0 c	0 a			2.0 b	2 7 b
Untreated	0 b	0 a	0 b	0 a	120 b	0 a			3 4 a	2.7 b

Values are means of four replicates in experiment 1 and five replicates in experiment 2; means within columns in each experiment followed by the same letter do not differ at $P \leq 0.05$ according to Duncan's multiple range test

[a] + Inoculated with 2 000 J2 of *M. arenaria* race 1 No nematodes added

[b] Less than 20 observations in at least one replicate

[c] – = No observation

with *M. arenaria* alone of experiment 1, fewer nematodes reproduced in air dried than in microwaved, and untreated soil ($P \leq 0.05$) (Table 3-3). This observation was not validated by experiment 2. Nematode reproduction in all three autoclaved soils did not differ from nematode reproduction in microwaved and air-dried soil collected from microplots without nematodes *P. penetrans*, except in the unmoculated microwave treatment of experiment 2 ($P \leq 0.001$, statistics not shown) (Tables 3-3 to 3-5). When no nematode inoculum was added, a very low level of nematode reproduction was observed only in untreated soil from plots infested with *M. arenaria* alone ($F \leq 0.001$) (Table 3-3) and in microwaved soil from unmoculated plots of experiment 2 (Table 3-5).

No females were infected with *P. penetrans* in all autoclaved soils and in soil treatments of nematode and *Pasteuria*-free soil (Tables 3-3 to 3-5). When soil was infested with nematodes, 100% of the females were infected in microwaved, air-dried, and untreated RKN + Pp soils of experiment 1 and 77% were infected in microwaved soil in experiment 2 (Table 3-4). A lower incidence of female infection occurred in the soil from microplots that had become infested with *P. penetrans*. Ten to thirty percent of females in microwaved, air-dried, or untreated RKN soil were infected with *P. penetrans* when nematodes were added (Table 3-3). When no nematode inoculum was added, infection rates of females in untreated RKN soil were 69 and 77%. However, females in unmoculated microwaved soil of nematode and *P. penetrans* free microplots were not infected by *P. penetrans*.

The effects of soil treatment on the fresh root weight were inconsistent (Tables 3-3 to 3-5). Peanut plants cultured in autoclaved or microwaved soil were not different or tended to developed larger root systems than those grown in air dried and untreated soils ($P \leq 0.05$). Inoculation level had no effect on the fresh root weight ($F \leq 0.05$) (Table 3-2).

TABLE 3.5 Effect of autoclaving, microwaving and air drying of soil maintained free of nematodes and *Pasteuria penetrans* on the nematode reproduction, percentage of females infected with *P. penetrans* and fresh root weights of peanut cv. Flarunner following inoculation with 0 or 2 000 second stage juveniles (J2) of *M. arenaria* race 1

Soil treatment	Nematode reproduction: Number of root galls per root system, Inoculation level		Number of egg masses per root system, Inoculation level		Number of eggs per root system, Inoculation level		% females infected (mean of 20 observations), Inoculation level		Fresh root weight (g), Inoculation level	
	+ [a]		+		+		+		+	
Experiment 1										
Autoclaved	588 a	0 a	513 a	0 a	118 500 a	0 a	0 a	[d]	4.9 a	4.0 a
Microwaved	402 a	0 a	406 a	0 a	130,900 a	0 a	0 a		4.7 a	4.1
Air dried	376 a	0 a	256 ab	0 a	72 000 a	0 a	0 a		4.7 a	3.8 b
Untreated	182 b	0 a	111 b	0 a	31,200 b	0 a	0 a		4.1 a	3.9 b
Experiment 2										
Autoclaved	363 a	0 b	303 a	0 b	88 500 a	0 b	0 a		3 b	4.3 a
Microwaved	375 a	3 a	306 a	2 a	86,000 a	380 a	0 a	0[b]a	4.3 a	3 b
Air dried	148 b	0 b	118 b	0 b	34 600 b	0 b	0 a		2.5 b	4.6 a
Untreated	432 a	0 b	382 a	0 b	88 000 a	0 b	0 a		3.3 b	2.8 b

Values are means of four replicates in experiment 1 and five replicates in experiment 2. Means within columns in each experiment followed by the same letter do not differ at $P \leq 0.05$ according to Duncan's multiple range test.

[a] + Inoculated with 2 000 J2 of *M. arenaria* race 1. No nematodes added.

[b] Less than 20 observations in at least one replicate.

[d] – No observation.

When no nematodes were added no nematode reproduction was noted in all but the untreated soils of RKN and RKN + Pp soil and in microwaved control soil (Tables 3-3 to 3 5, statistics not shown) Nematode reproduction generally did not differ between inoculated and uninoculated treatments in microwaved air dried and untreated soils of RKN and RKN + Pp plots (except in the microwaved and untreated soil of RKN plots), but was higher in all inoculated, control soils and following the autoclave treatment ($P \leq 0.05$)

Discussion

An increase in treatment time or in soil moisture content resulted in an increasing effect of microwaving soil on soil microorganisms. The response of soil fungi to an increase in soil moisture content does not agree with Ferriss s (1984) observations on microorganisms He reported that soil temperature and the effect on reduction of populations of microorganisms correlated positively with MW treatment time and negatively with soil moisture content Moisture levels approaching field capacity were required to kill spores of root-pathogenic fungi by microwave heat treatment (Baker and Fuller 1969) The percentages of soil moisture of loamy soils used in Ferriss' (1984) studies exceeded by far the soil moisture contents of soils used in this study Loamy soils would probably require an extended period of microwave treatment to approach an effect similar to that attained on sandy soils The different results also may be explained by the fact that sandier soils cool faster than loamy soils after microwaving (Ferriss, 1984), hence, fungal propagules may have been affected by the duration of the elevated temperatures rather than by the temperature peak

Endospores of *P penetrans* are reportedly resistant to high temperatures (Stirling et al., 1986, Williams et al., 1989), however, when endospores were exposed to 80 °C for 30 minutes their development was impeded (Dutky and Sayre 1978). More recent studies showed that attachment was close to minimum at 60 °C or higher, but it was not completely prevented at 100 °C for 5 hours per day over 10 days (Freitas 1997). In this study, a microwave treatment time of 3 minutes for a 1-kg sample of soil containing nearly 7% moisture heated the soil to 70 to 75 °C for less than 1 minute. Following the microwave treatment the number of endospores attached per juvenile was reduced to a variable degree, but was not lower than four endospores per juvenile. This effect was tolerable because the germination of a single spore is enough to create infection in the female *Meloidogyne* (Stirling, 1984) and hence, sufficient to be used to confirm nematode suppression by *P penetrans*. One hundred percent infection of females occurred after the microwave soil treatment, indicating that *P penetrans* was able to survive the microwave treatment and suppress the nematode population in the pot experiments.

Although methods to quantify facultative and obligate nematophagous fungi in soil have been developed, they do not always give satisfactory results (Dackman et al., 1987). Propagules of saprophytic and nematophagous fungi are of similar structure (Barron, 1992), therefore they are likely to withstand similar amounts of microwave radiation. If the results of the dilution plating technique were extrapolated to the survival of nematophagous fungi microwaving soil for 3 minutes per kg of soil had the potential to selectively eliminate this group of microorganisms. This allows for the separation of the antagonistic effects of fungi and the bacterial parasite. Microwaving soil 3 minutes for each kilogram of soil was chosen to be the most appropriate microwave treatment to be used in the suppressive soil test. The treatment does not leave a nematicidal residual effect,

as observed with soil applications of 40% formaldehyde (Dickson et al., 1994 Kerry et al., 1982) and benomyl (Hoestra 1976) treatment

The numerical difference in attachment of endospores to J2 between the two microwave studies on water content and treatment time may be due to the fact that the dried soil containing *P penetrans* was stored for 8 weeks between the studies thus affecting the ability of endospores to attach to nematodes. A minimum of 3 days moisture incubation (Brown and Nordmeyer, 1985) before the application of soil treatments and nematode inoculum may have enabled more endospores to attach.

Attachment by *P penetrans* was detected on J2 exposed to soil from RKN and RKN + Pp microplots. Nematode reproduction was consistently inhibited in the presence of viable *P penetrans* endospores in these soils. Endospores in both soils existed in densities at which plant infectivity by the J2 was reduced which protected the root systems from being severely galled Similar results were found when J2 were encumbered with 15 or more endospores of *P penetrans* and used to inoculate tomato (Brown and Smart, 1985, Davies et al , 1988 1990 Seil and Hansen 1987 Stirling 1984) The infection of *M arenaria* by *P penetrans* resulted in low fecundity This supports earlier observations that infection by *P penetrans* causes sterility or reduced fecundity in females (Mankau, 1980a Minton and Sayre, 1989) however the number of isolated females was high enough to statistically confirm 100% infection by *P penetrans* only from soils infested with *M arenaria* and *P penetrans* used in experiment 1 Additional tests with soils containing a more diluted *P penetrans* population density may be needed to confirm the nematode suppression by *P penetrans* Temperature has been reported to affect nematode mobility (Bird and Wallace, 1965) attachment of endospores to J2 (Freitas et al , 1997, Hatz and Dickson, 1992; Stirling 1981) and the development of *P penetrans in vivo* (Hatz and Dickson 1992 Nakasono et al., 1993, Sarracin et al , 1997) The constant

temperatures of the growth room used for experiment 1 probably favored the development of *P. penetrans*.

Although in this study the effect of fungi and *P. penetrans* could not be evaluated separately, an additive nematode suppression would be expected to cause differences in nematode reproduction between the treatments of microwaving or air drying of RKN and RKN + Pp soils. The lowest numbers of root galls, egg masses, and eggs were observed following air-drying of RKN soil in experiment 1 and of RKN + Pp soil in experiment 2. However, since these effects could not be observed when replicated, fungi appeared to play only a minor role in nematode suppression. These results supported the hypothesis that *P. penetrans* is the main contributor to the suppressiveness in soils from both the RKN and RKN + Pp microplots.

In the suppressive soil test, the microwave treatment was helpful because it reduced fungi that might also be a factor in suppressive soils. However, the endospore density in RKN + Pp soil was so high that it greatly suppressed the plant infectivity by endospore-encumbered J2, and the female infection rate could not be determined. It cannot be ruled out that the nematode suppression was due to other factors than parasitism by *P. penetrans*, however, compared to the control soil, there were only minor differences between the test results of the RKN and the RKN + Pp soil comprising nematode suppression observed in Chapter 2. Hence, it can be concluded that the suppressiveness of the soils is of similar nature.

At the time the soil for this test was collected from the microplots, nematodes of RKN + Pp plots had been suppressed for 3 years. This study demonstrated that endospores of *P. penetrans* survived 3 years in the absence of a host and successfully suppressed a reinoculated nematode population. Although it is not known exactly, dormant endospores are probably able to survive for many more years. *Pasteuria* spp.

differ from successful natural enemies of insects, which usually require a constant food supply to maintain their population density (Bennett 1974) The absence of a host nematode appears to have little impact on the survival of *P. penetrans*, which may be a leading attribute for the organism in suppression of nematodes under various conditions

CHAPTER 4
POPULATION DEVELOPMENT OF *MELOIDOGYNE* SPP AND *PASTEURIA PENETRANS* AS AFFECTED BY CULTURAL PRACTICES IN TOBACCO

Introduction

The endospore-forming bacterial parasite, *Pasteuria penetrans* (Thorne) Sayre & Starr, is widely distributed in agricultural soils throughout the world (Sayre and Starr 1988, Stirling 1991) and contributes to natural and induced nematode control, especially of *Meloidogyne* spp (Bird and Brisbane 1988, Brown et al. 1985, Channer and Gowen, 1988, Chen et al. 1994, 1996b, 1997c, Dickson et al. 1994, Kerry 1990, Mankau, 1980a, 1980b, Minton and Sayre 1989, Oostendorp et al., 1991a, Weibelzahl-Fulton et al. 1996). In most observations the suppression of *Meloidogyne* spp by *P. penetrans* occurred after long term monoculture of susceptible hosts in association with *Meloidogyne* spp (Bird and Brisbane, 1988, Chen et al. 1994, Mankau, 1980a, Minton and Sayre 1989, Chapter 2). *Pasteuria penetrans* has great potential for integration with other cultural or nematode management practices (Brown and Nordmeyer, 1985, Freitas 1997, Maheswari et al. 1988, Oostendorp et al. 1991a, Stapleton and Heald, 1991, Taoritzakakis and Gowen, 1994a, Walker and Wachtel 1989). Pathogenicity and virulence of *P. penetrans* can vary among different bacterial isolates (Channer and Gowen 1992, Davies et al. 1994, Oostendorp et al., 1990), and knowledge about their biology is required to effectively include the organism into nematode management strategies (Stirling 1991).

A mixed population of root knot nematodes, *Meloidogyne incognita* (Kofoid & White) Chitwood, *M. javanica* (Treub) Chitwood, and *M. arenaria* (Neal) Chitwood, was

observed to damage tobacco grown in a field at the University of Florida Green Acres Agronomy Farm, Alachua County *Pasteuria penetrans* and several species of nematophagous fungi were isolated from the site in 1991 (Chen et al., 1994) *Pasteuria penetrans* was identified to be suppressive to the mixed populations of root-knot nematodes and significant changes in the density of *Meloidogyne* spp were observed with inorganic nitrogen rates, cover crops, and tobacco cultivars (Chen et al., 1994)

The objectives of this study were to test the pathogenicity of the naturally occurring *P penetrans* population to *M incognita* and *M javanica* in the laboratory, and to determine the effects of nitrogen fertilizer, autumn cover crop and tobacco cultivars on the population density development of *Meloidogyne* spp and *P penetrans* in the tobacco field

Materials and Methods

In the summer 1992 a random sample of approximately 30 roots was collected from the tobacco site The roots were washed free of soil cut into 2- to 3-cm pieces and mixed thoroughly Forty females and accompanying egg masses of *Meloidogyne* spp were dissected from randomly selected root pieces and the females were placed individually on an eye glass in a drop of deionized water The egg masses were treated with 0 5% NaOCl for 1 minute, washed with sterile water three times, placed individually into 2.5 ml microfuge tubes containing 1 ml of deionized water and incubated at room temperature The females were cut open at the neck region to release the body contents, which were then examined for mature endospores of *P. penetrans* The nematode species was identified by optical examination of 20 perineal patterns

Nematode Populations

One week after isolation from roots 5 ml of water containing approximately 250 second-stage juveniles (J2) hatched from egg masses obtained from three *Pasteuria*-free females of either *M. incognita* or *M. javanica* were placed into five 2-cm-deep holes around the stems of two 4-week-old tomato (*Lycopersicon esculentum* L. cv. Rutgers) plants and maintained in the greenhouse. After 60 days, the root systems were harvested and washed free of soil. Eggs of *M. incognita* and *M. javanica* were extracted from the infected tomato roots (Hussey and Barker, 1973), hatched by the Baermann method (Rodríguez-Kábana and Pope, 1981), and used for experiments as 1- to 4-day-old J2 or inoculated to ten 6-week-old tomato plants, and maintained in the greenhouse.

Pasteuria penetrans Isolates

One week after their isolation from roots, approximately 250 J2 of three *Pasteuria*-free females of both *M. incognita* and *M. javanica* were exposed to mature endospores of *P. penetrans* harvested from one female of the same species. After 24 hours incubation in water at room temperature (26 °C), the average number of endospores attached to 20 randomly selected J2 was determined with an inverted microscope at ×400 magnification. The endospore encumbered J2 were suspended in 5 ml of water and then placed into five 2-cm-deep holes around the stems of two 4-week-old tomato (*Lycopersicon esculentum* L. cv. Rutgers) plants and maintained in the greenhouse. After 60 days, the root systems were harvested and washed free of soil.

Root systems were enzymatically digested in Pectinol (Genencor, South San Francisco, CA) at room temperature for 48 hours. Mature globose females were hand

picked from the softened roots and air dried on an eye glass. After 3 days the nematode bodies were rehydrated for 8 hours and macerated in a glass tissue grinder. The number of endospores was counted with the aid of a hemacytometer, and their concentration was adjusted to 1×10^5 endospores per ml water.

Laboratory Experiment

Attachment studies were conducted using the centrifuge technique (Hewlett and Dickson 1993). A 0.1-ml sample of the endospore-water suspension and 0.1 ml of a suspension of 2,000 J2 of *M. incognita* or *M. javanica* per ml of water were placed in a 0.25-ml previously silanized microfuge tube and centrifuged at 9,500g for 2 minutes using a microfuge. Each attachment study was replicated five times. The content of the microtube was stirred and placed on a glass slide with a pipette. The number of endospores per juvenile was determined for 20 randomly selected nematodes per replicate. Thereafter, the endospore-encumbered nematodes of both species were suspended in 5 ml of water and inoculated to tomato plants as described above.

Field Experiment

The site had been planted to tobacco in the same plots for seven consecutive years. The 3 × 2 × 2 factorial treatment design included three autumn cover crops (hairy indigo *Indigofera hirsuta* L., forage sorghum, *Sorghum* spp., and weed fallow), two inorganic nitrogen fertilizers (89 and 158 kg of ammonium nitrate/ha), and two tobacco cultivars (Coker 371-Gold, which is susceptible to *M. incognita*, *M. javanica*, and *M. arenaria*, and Northrup King K-326, which is resistant to *M. incognita* but susceptible to *M. javanica* and

M. arenaria). Hairy indigo and forage sorghum were planted in August and plowed under in November of each year. Rye was planted over the entire field in early December to serve as a winter cover crop and plowed under in March. Tobacco seedlings were transplanted on 27 March and on 2 April of 1992 and 1993, respectively. The final harvest was taken on 4 August and 11 August of 1992 and 1993, respectively. Preplant fertilization included a broadcast application of 1,800 kg of 6-6-18 (N-P-K) and 340 kg of 15-0-14 (N-P-K) of sodium potassium nitrate per hectare. At the final cultivation in late April, granular ammonium nitrate was applied adjacent to the plant stems. The plots were arranged in complete randomized blocks and replicated four times. Each block consisted of one row with a row spacing of 1.2 m and a length of 12 m.

A 12-core soil sample was taken with a cone-shaped auger (2.5-cm diameter) from the root rhizosphere (20 cm deep) of each plot on 2 April, 9 June, and 28 August of 1992 and on 2 April, 24 June, and 14 August of 1993. The soil was mixed, sampled for soil moisture determination (samples of 2 April 1992 and 1993), and processed using centrifugal-flotation method (Jenkins, 1964). The number of J2/100 cm^3 of soil, the rate of attachment of *P. penetrans*, and the number of endospores attached per 20 randomly selected J2 were determined using an inverted microscope at ×400 magnification. Root samples were collected after the final tobacco harvest. 20 females were dissected randomly from each sample, crushed on a glass slide, and checked for infection by *P. penetrans* with a compound microscope at ×1000 magnification. Effects of the autumn cover crop were determined for the 1992 season only.

Results

Laboratory Experiment

Meloidogyne incognita and *M. javanica* were identified from tobacco root samples which was thereafter confirmed by Chen et al. (1994). After 24 hours of incubation of the original *P. penetrans* isolates with J2 of either species at room temperature, 87% of J2 of *M. incognita* and 82% of J2 of *M. javanica* were attached, with an average number of 3.8 and 6.7 endospores per juvenile, respectively (data not shown). With the exception of one attachment test conducted with the *P. penetrans*-isolate from *M. javanica*, the number of endospores per juvenile of *M. incognita* was higher than or not different than the number of endospores attached per juvenile of *M. javanica* ($P \leq 0.05$) (Tables 4-1 and 4-2). Attachment of the *M. javanica*-isolate was higher on *M. incognita* than on *M. javanica* after the bacterial parasite was grown on *M. incognita* for two consecutive generations ($P \leq 0.05$) (Table 4-2). However, endospore attachment of the *M. incognita* isolate after three generations on *M. javanica* was either maintained or reduced (Table 4-1). The percentages of J2 attached with endospores were between 72 and 100% (Tables 4-1 and 4-2). In the first and second generation attachment tests, the number of endospores per J2 and the percentage of encumbered J2 followed the same trends. In the third generation attachment tests, the percentages of J2 with attached endospores generally were not different.

Field Experiment

The soil moisture content ranged from 6.1 - 11.8% soil moisture in 1992 and 6.4-11.1% in 1993. The number of root-knot nematode juveniles in the soil and the galling

TABLE 4-1 Attachment pattern on second stage juveniles (J2) of *Meloidogyne javanica* and *M. incognita* over three generations of *Pasteuria penetrans* isolate P-110 from *M. incognita* infecting tobacco

1st generation			2nd generation			3rd generation		
Test nematode	Number of endospores/ juvenile	% attachment	Test nematode	Number of endospores/ juvenile	% attachment	Test nematode	Number of endospores/ juvenile	% attachment
M. incognita[a]	15.3 a	100 a	*M. incognita*[b]	9.7 a	94 a	*M. incognita*	4.8	100
						M. javanica	3.9	100
			M. javanica[b]	2.0 b	66 b	*M. incognita*	21 a	86
						M. javanica	7 b	75
M. javanica[a]	6.2 b	78 b	*M. incognita*[b]	6.9	72	*M. incognita*	8.0	81
						M. javanica	3.5	90
			M. javanica[b]	3.2	77	*M. incognita*	1.8	89
						M. javanica	1.4	93

Means followed by no letter within a column of two observations were not different according to Student's *t* test. Values are means of five replicates. 20 randomly selected J2 were observed per replicate.

[a] endospores produced in this nematode population were used in the 2nd generation attachment test.

[b] endospores produced in this nematode population were used in the 3rd generation attachment test.

TABLE 4-2. Attachment pattern on second-stage juveniles (J2) of *Meloidogyne javanica* and *M. incognita* over three generations of *Pasteuria penetrans* isolate P-120 from *M. javanica* infecting tobacco

1st generation			2nd generation			3rd generation		
Test nematode	Number of endospores/ juvenile	% attachment	Test nematode	Number of endospores/ juvenile	% attachment	Test nematode	Number of endospores/ juvenile	% attachment
M. incognita[a]	4 8	93	*M. incognita*[b]	17 4 a	100 a	*M. incognita*	20 8 a	100
						M. javanica	14 2 b	100
			M. javanica[b]	2 5 b	88 b	*M. incognita*	2 4	91
						M. javanica	2 5	84
M. javanica[a]	8 3	97	*M. incognita*[b]	1 4 b	73 b	*M. incognita*	1 4	74
						M. javanica	1 5	72
			M. javanica[b]	10.2 a	100 a	*M. incognita*	12 6	100
						M. javanica	8 0	100

Means followed by no letter within a column of two observations were not different according to Student's *t* test. Values are means of five replicates; 20 randomly selected J2 were observed per replicate.

[a] endospores produced in this nematode population were used in the 2nd generation attachment test.

[b] endospores produced in this nematode population were used in the 3rd generation attachment test.

indices were generally higher in 1992 than in 1993 (statistics not shown) (Table 4-3). At planting and after harvest in 1992, the root-knot nematode density was higher in plots with the history of the higher rate of inorganic nitrogen than in those that received the low nitrogen rate ($P \leq 0.05$). This effect was not observed in 1993. Throughout 1992 and 1993, the population density of J2 in the soil was increased by both tobacco cultivar histories, but no difference was observed between cultivars in 1992 and most of 1993. However, a higher nematode population density under the resistant tobacco cultivar than under the susceptible cultivar was revealed on the last sampling in 1993. The number of J2 was higher with weeds than hairy indigo or sorghum at harvest in 1992. At mid-season of 1992, but not in 1993, an interaction between cultivar and autumn cover crop was found to affect the number of J2 in the soil (Table 4-3). The nematode population density was lowest in NK 326 plots that followed the forage sorghum cover crop (data not shown). This observation was not validated by repeat in 1993.

In 1992, interactions between the inorganic nitrogen fertilizer levels and the tobacco cultivar histories affected the number of J2 in the soil at planting and after tobacco harvest ($P \leq 0.05$) (Tables 4-3 and 4-4). At planting and after harvest, the higher rate of ammonium nitrate fertilizer resulted in a greater population density of J2 in the root rhizosphere of NK 326 than in Coker 371 Gold. As opposed to NK 326, the number of nematodes in the root rhizosphere of Coker 371-Gold was not increased by the higher level of fertilizer (Table 4-4).

Root galling of Coker 371-Gold was less than that of NK 326 ($P \leq 0.001$ for 1992, $P \leq 0.05$ for 1993). In 1992, root galling on tobacco was less in plots following hairy indigo and forage sorghum than in plots following weeds ($P \leq 0.01$), but no observations were made in 1993 to validate the effects of the cover crops. The galling indices were not affected by the nitrogen treatments; however, an interaction between the inorganic nitrogen

TABLE 4-3 Population density development of *Meloidogyne* spp. in 1992 and 1993 as determined by the number of second-stage juveniles (J2) in the soil, and the galling indices of roots of two tobacco cultivars treated with two inorganic nitrogen rates, and three autumn cover crop treatments

Treatment	Treatment level	J2 per 100 cm³ of soil						Galling index[a]	
		1992			1993				
		2 April	9 June	28 August	2 April	24 June	14 August	1992	1993
Nitrogen	89 kg/ha	8 b	72	308 b	3	20	234	2 9	1 6
	158 kg/ha	16 a	41	657 a	5	34	240	2 8	1 9
Cultivar	Coker 371-Gold	9	38	473	4	31	138 b	2 4 b	1 2 b
	NK 326	15	75	491	4	23	336 a	3 3 a	2.3 a
Cover crop	Weeds	11	56	653 a			-	3 3 a	
	Hairy indigo	9	44	270 b		-	-	2 7 b	-
	Forage sorghum	15	69	372 b		-		2.6 b	
ANOVA	Block	NS	NS	NS	NS	NS	NS	***	***
	Nitrogen (N)	**	NS	*	NS	NS	NS	NS	NS
	Cultivar (C)	NS	NS	NS	NS	NS	*	***	*
	Cover crop (Cc)	NS	NS	*	-	-		**	
	N x C	*	NS	*	NS	NS	NS	*	*
	N x Cc	NS	NS	NS	-			NS	
	C x Cc	NS	*	NS	-		-	NS	
	N x C x Cc	NS	NS	NS		-	-	*	-

Data are means of main effects. The number of J2 were transformed with $\log_{10}(x+1)$ before being subjected to ANOVA. Means within treatments of each date followed by the same or by no letter do not differ at $P \leq 0.05$ according to ANOVA for nitrogen and cultivar, or Duncan's multiple-range test for cover crop.

* ** *** represent $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively. NS = not significant at $P \leq 0.05$.

- = no observation.

[a] The galling index scale: 0 = no galls, 1 = 1-10, 2 = 11-20, 3 = 21-55, 4 = 56-80, 5 = 81-100% of roots galled.

TABLE 4-4 Interaction between inorganic nitrogen fertilizer levels and tobacco cultivar history and their effects on the number of second-stage juveniles (J2) of *Meloidogyne* spp. in 100 g of soil collected at planting and after the final harvest in 1992

	Number of J2 per 100 g of soil			
	2 April		28 August	
Cultivar history	89 kg N	158 kg N	89 kg N	158 kg N
Coker 371-Gold	8 a A	9 b A	477 a A	469 b A
NK 326	8 a B	23 a A	140 b B	844 a A

The values were transformed with $\log_e (x + 1)$ before being subjected to ANOVA; the same lower case letters in columns or upper case letter in rows for the same sampling dates indicate no significant difference at $P \leq 0.05$ according to ANOVA.

TABLE 4-5 Interaction between inorganic nitrogen fertilizer levels and tobacco cultivar history, and their effects on the root-galling index in 1992 and 1993

	Root galling index[a]			
	1992		1993	
Cultivar history	89 kg N	158 kg N	89 kg N	158 kg N
Coker 371-Gold	2.7 a A	2.2 b A	1.0 b A	1.4 b A
NK 326	3.1 a A	3.4 a A	2.2 a A	2.4 a A

The values were transformed with arcsin ($\sqrt{x}$) before being subjected to ANOVA; the same lower case letters in columns or upper case letter in rows indicate no significant difference at $P \leq 0.05$ according to ANOVA.

[a] The galling index scale: 0 = no galls, 1 = 1-10, 2 = 11-20, 3 = 21-55, 4 = 56-80, 5 = 81-100% of roots galled.

fertilizer and the tobacco cultivar history was found to affect the galling indices ($P \leq 0.001$) (Tables 4-3 and 4-5). The high rate of ammonium nitrate resulted in more severe root galling on NK-326 than on Coker 371-Gold in both years ($P \leq 0.05$) (Table 4-5). This statement is valid for the low level of nitrogen in 1992 but not for 1993. In comparison with a root galling index of 3.8 in the weed fallow plots, the severity of root galling decreased to 2.0 and 1.7 after Coker 371-Gold plots were covered with hairy indigo or forage sorghum, respectively (data not shown). Galling indices differed between the blocks in both seasons ($P \leq 0.001$) (Table 4-3).

A high percentage of the J2 had endospores of *P. penetrans* attached (Table 4-6). The percentage attachment and the number of endospores attached per juvenile remained fairly constant over the six sampling dates. In June and August of 1992, and in August 1993, the percentages of J2 with attached endospores were higher in the root rhizosphere of Coker 371 Gold than in NK 326 ($P \leq 0.05$). The autumn cover crops affected the percentage of attached J2 only on the first sampling date of the first year. At that time more J2 were encumbered with endospores after an autumn cover with weeds or hairy indigo than after forage sorghum. No effect of the inorganic nitrogen fertilizer was observed on the percentage of J2 with attached endospores of *P. penetrans* or the number of endospores per juvenile. The percentage of females infected by *P. penetrans* was not affected by the treatments. Variations among blocks affected endospore attachment and the female infection rate at various sampling dates.

An interaction between tobacco cultivar history and autumn cover crop revealed that in the root rhizosphere of NK 326, more J2 were encumbered with endospores after weed and hairy indigo autumn cover than after forage sorghum ($P \leq 0.05$) (data not shown). There was an interaction among the nitrogen levels, the tobacco cultivar history, and the autumn cover crops affecting the percentage of J2 attached with

TABLE 4-6 Percentage of second-stage juveniles (J2) of *Meloidogyne* spp. with attached endospores of *Pasteuria penetrans*, average number of endospores attached per juvenile in the soil, and percentage of *P. penetrans*-infected females collected from two tobacco cultivars in a field treated with two nitrogen fertilizer rates and three autumn cover crops in 1992 and 1993

		Percentage of J2 with attached endospores						Number of endospores per juvenile						% infected females	
		1992			1993			1992			1993				
Treatment	Treatment level	2 April	9 June	28 August	2 April	24 June	14 August	2 April	9 June	28 August	2 April	24 June	14 August	1992	1993
Nitrogen	89 kg/ha	24	55	64	66	42	73	0.9	3.1	4.3	1.4	0.4	1.8	69	73
	158 kg/ha	23	62	55	29	50	64	1.8	3.4	3.3	0.6	0.6	1.0	52	56
Cultivars	Coker 371 Gold	23	67 a	65 a	55	53	78 a	1.3	3.3	4.0	1.1	0.5	2.1 a	66	68
	NK326	24	51 b	54 b	40	38	58 b	1.4	3.1	3.5	0.8	0.4	0.8 b	55	61
Cover crops	Weeds	37 a	63	60				2.0	3.8	4.3	-			59	
	Hairy indigo	24 a	66	65				1.1	2.4	4.1				67	
	Forage sorghum	8 b	48	54				1.0	3.5	3.0				55	
ANOVA	Block	NS	*	NS	NS	**	**	NS	NS	*	NS	NS	*	**	*
	Nitrogen (N)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Cultivar (C)	NS	*	**	NS	NS	**	NS	NS	NS	NS	NS	*	NS	NS
	Cover crop (Cc)	**	NS	NS				NS	NS	NS				NS	
	N x C	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	N x Cc	NS	NS	NS				NS	NS	NS					
	C x Cc	*	NS	NS				NS	NS	NS					
	N x C x Cc	**	NS	NS				NS	NS	NS					

Data are means of main effects. The percentages were transformed with arcsin ($\sqrt{x}$) before analysis and the average numbers of endospores per juvenile were transformed with $\log_{10}(x+1)$ before being subjected to ANOVA. Means within treatments at each date followed by the same or by no letter do not differ at $P \leq 0.05$ according to ANOVA for nitrogen and cultivar or Duncan's multiple range test for cover crop

* ** *** represent $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively. NS = not significant at $P \leq 0.05$

– no observation

TABLE 4-7 Interaction amoeg inorganic nitrogen fertilizer levels tobacco cultivar history and cover crops and their effects oo the percentage of second stage juveniles (J2) encumbered with endospores of *Pasteuria penetrans* at planting in 1992

		Percentage of J2 encumbered with endospores	
Cultivar history	Cover crop	89 kg N	158 kg N
Coker 371-Gold	Weed fallow	34 a A	34 a A
	Hairy indigo	6 b A	27 a A
	Forage sorghum	15 b A	12 a A
NK-326	Weed fallow	32 b A	46 a A
	Hairy indigo	46 a A	17 b B
	Forage sorghum	1 c A	4 b A

The values were transformed with arcsie ($\sqrt{x}$) before subjected to ANOVA and Duncan's multiple-range test The same lower case letters in columns within each cultivar history or upper case letter in rows indicate eo significant differeeee at $P \leq 0.05$ according to Duncan's multiple range test or Student *t*-test respectively

endospores at the time of planting (Table 4-6 and 4-7) In NK 326 plots under hairy indigo the low rate of ammonium nitrate resulted ie the highest eedospores attachment per juvenila In comparisoo with weeds and sorghum ($P \leq 0.01$) however at the high rate of fertilizer the percentage of attachment was highest after weed fallow The trends were not observed io 1993 The highest endospores attachment per juvenile in Coker 371-Gold was observed at the low rate of eitrogen after weed fallow, but there was no differenc between the nitrogen levels

Discussion

Over a time period of 2 years in a loeg-term field experiment with tobacco, the nematode population densities and root-galling indices declined, whereas the *P*

penetrans population densities increased. The relatively high percentages of females with endospores verifies that *P. penetrans* was established and impacted the root-knot nematode populations. The two isolates of *P. penetrans* were similar or sometimes more virulent to *M. incognita* than to *M. javanica*, however this experiment was not repeated for validation.

Multiple years of Coker 371-Gold favored endospore build-up in the soil. These observations are in contrast with results from a preceding season (Chen et al. 1994), in which nematode populations densities in the root rhizosphere of NK-326 were lower than those of Coker 371-Gold, and the percentage of endospore encumbered J2 was not different or slightly higher in NK 326 than in Coker 371-Gold plots. This trend may have resulted from the suppression of predominantly *M. incognita*, and the build-up of mainly *M. javanica*, which was less susceptible to attachment by *P. penetrans* endospores under laboratory conditions.

Inorganic nitrogen urea and their analogs are known to have nematicidal effects (Rodriguez-Kábana, 1986; Rodriguez Kábana et al., 1981; Spiegel et al., 1982; Talavera et al., 1984) and to inhibit the development of root-knot nematodes in plant roots by preventing the formation of feeding sites (Glazer and Orion 1984). Results observed in this study suggest that this mechanism may be of little or no importance for tobacco under field conditions. During the 1992 season, but not in the 1993 season, the tobacco cultivar with resistance to *M. incognita* supported a higher nematode population than the susceptible cultivar when receiving the high level of nitrogen.

In early season effects by the weed fallow and hairy indigo autumn cover crop may enhance the percentage of endospore-encumbered J2 in the soil. This trend was observed in 1992 but could not be validated in 1993. Although hairy indigo is usually planted to suppress root-knot nematode populations, some nematode reproduction can

occur (Dominguez et al , 1985 McSorley et al., 1994) and contribute to the build up of *P penetrans* endospores.

There were outcroppings of clay pockets across the field These small areas hold more water, thereby leading to variations in soil moisture Although not mapped, its existence probably effected galling indices, the percentage of J2 with attached endospores, and the percentage of females infected with *P penetrans* The development of *Meloidogyne* spp. is affected by fine textured soils (Windham and Barker 1986) and the development of both the nematode and *P penetrans* was slower when soil moisture was maintained at field capacity (Davies et al , 1991) The efficacy of the bacterial parasite is known to depend on the density of its host nematode (Stirling, 1991) All this suggests that oxygen depletion in wet soil inhibits root development and respiration, supports the establishment of root pathogens, lowers the reproductive rates of the nematode, and, hence, hampers the increase of the *P penetrans* endospore population densities in soil

It is not clear whether the resistant tobacco cultivar induced a shift in the composition of the root-knot nematode population Supporting laboratory studies suggested that attachment of the *M javanica*-isolate to *M incognita* increased after the bacterial parasite was grown on *M incognita* for two consecutive generations thus, the *P penetrans* population present in the field appears to be capable of adjusting to a possible change in the nematode population. If there is time for natural selection to take place, it may be possible for new strains of *Pasteuria* spp to evolve that are better fitted to the local environment or more virulent to the shifting nematode population. This also was observed by others (Channer and Gowen 1992 Oostendorp et al , 1990) As previously reported, *P penetrans* has maintained itself in situations where nematode populations were altered by a change in cropping practice (Stirling 1991)

CHAPTER 5
SUPPRESSION OF *MELOIDOGYNE INCOGNITA* AND *M. JAVANICA* BY *PASTEURIA PENETRANS* IN FIELD SOIL

Introduction

Pathogen suppressive soils are defined (Baker and Cook, 1982) as soils in which the pathogen does not establish or persist establishes but causes little or no damage or establishes and causes disease (conducive soil) but thereafter the disease is reduced even though the pathogen persists in the soil. Soils suppressive to plant pathogens may occur naturally as an inherent characteristic of the physical, chemical, or biological structure of a particular soil. Suppressiveness also may be induced by some agronomic practices such as planting a crop or adding organic or nutritional amendments which change the microflora. Induced suppressiveness apparently develops as a result of the buildup of antagonists in response to a high pathogen population (Baker and Cook, 1982).

The endospore-forming bacterial parasite, *Pasteuria penetrans* (Thorne) Sayre & Starr, is widely distributed in agricultural soils throughout the world (Sayre and Starr, Stirling, 1991c, 1988) and contributes to natural and induced nematode control, especially of *Meloidogyne* spp. (Bird and Brisbane, 1988; Brown et al., 1985; Channer and Gowen, 1988; Chen et al., 1996b; Dickson et al., 1994; Kerry, 1990; Mankau, 1980a, 1980b; Minton and Sayre, 1989; Oostendorp et al., 1991a). The suppression of *Meloidogyne* spp. by *P. penetrans* occurred mostly after long-term monoculture of susceptible hosts with

Meloidogyne spp. (Bird and Brisbane 1988, Chen et al., 1994, Mankau, 1980a, Minton and Sayre, 1989)

In 1994 a site in Alachua County Florida that was suppressive to *Meloidogyne* spp. was identified (Chen et al., 1994, Chapter 4). The field had been planted continuously for 7 years to flue-cured tobacco (*Nicotiana tabacum* L.). No nematicides or other nematode management practices were applied to the site. Root-knot damage was initially severe and caused by a mixed population of *M. incognita* race 1 (Kofoid & White) Chitwood and *M. javanica* (Treub) Chitwood. Yield reductions decreased over the years to a point where there was little economical loss (Whitty, personal communication). Endospores of *P. penetrans* were found in mature females and attached to second-stage juveniles (J2) of both nematode species from samples collected throughout the field, and several species of fungi were isolated from J2 and egg masses (Chen et al., 1994). The objective of this study was to determine the role of *P. penetrans* in suppressing *M. incognita* and *M. javanica* in soil from this tobacco field.

Materials and Methods

Soil Treatments

The field site was located at the University of Florida Green Acres Agronomy Farm, Alachua County. Approximately 90 liters of soil, a loamy, siliceous, hyperthermic Grossarenic Paleudults with 90% sand, 4% silt, 6% clay, and 2.5% organic matter, was collected with a bucket auger (10-cm diameter) in March 1993 (greenhouse experiment 1) and in July 1993 (greenhouse experiment 2) from the tobacco field. The soil was passed

through a 5-mm aperture sieve, mixed, and divided into four subsamples of 22 liters each. The subsamples were either autoclaved, microwaved, air dried, or left untreated.

Autoclave heating treatment. Sterilized, 15-cm-diameter clay pots were filled with soil (ca. 1 liter/pot), covered with aluminum foil, and placed in an autoclave. After the first heat treatment for 1.5 hours at 55 kPa, the pots were removed from the autoclave. The treatment was repeated after pots and soil had cooled to room temperature.

Microwave heating treatment. For the microwave heating treatment, 1 kg lots of soil were placed in even layers in open plastic bags and heated in a microwave oven for 3 minutes (Chapter 3) at full power (1,500 W, 2,450 Mhz), which caused the temperature to rise to about 75 °C.

Air drying treatment. The 22 liters of soil for the air drying treatment were collected 2 weeks prior to the collection of soil for the autoclaving, microwaving, and untreated soil treatments. The soil was placed in 3- to 5-cm-thick layers on plastic trays and stored for 2 weeks in the greenhouse to eliminate or reduce the natural nematode population.

Untreated soil. This soil remained as collected from the field.

Laboratory Experiments

One day after the soil was treated, a dilution plating technique was employed to assay for selected fungi (Johnson and Curl, 1972). One gram of the mixed soil from each soil treatment was dispersed and constantly agitated in 200 ml of sterilized water. Under sterile conditions 1 ml of the soil solution was plated on potato dextrose agar (PDA, Difco

Laboratories Detroid MI) containing 100 mg streptomycin, 50 mg chlortetracycline and 1 0 ml of tergitol/liter of medium respectively (Steiner and Watson, 1965) Six replicates were plated for each soil treatment After 3 days of incubation the fungal colony forming units (cfu)/g of soil were determined.

A soil bioassay (Oostendorp et al 1991a) used *Meloidogyne incognita* race 1 as the host to determine the presence of *P penetrans* A sample of 50 g of dry soil from each treatment was placed in a petri dish, and replicated six times for the bioassay The soil water content was adjusted to 100% field capacity to increase the rate of endospore attachment, and the dishes were left uncovered at room temperature (Brown and Nordmeyer, 1985) After 3 days 500 J2 of the test nematode were added and the moisture level was adjusted to 50% of field capacity The J2 were extracted by centrifugal flotation 2 days later (Jenkins, 1964) and the number of endospores per juvenile was determined with the aid of an inverted microscope for 20 randomly selected J2 per replicate.

Greenhouse Experiment

Treatments were arranged in a 2 × 2 × 4 factorial design that included four soil treatments (as listed above) inoculum levels of 0 and 2 000 J2 of *M incognita* race 1/pot and tobacco cultivars Coker 371 Gold (susceptible to both *M incognita* and *M javanica*) and Northrup King K 326 (resistant to *M incognita* but susceptible to *M javanica*) The soil from each treatment was placed into sterilized 15-cm-diameter clay pots (ca. 1 liter/pot). Treatments were replicated six times in the first experiment and five times in the second experiment The moisture level was adjusted to approximately 50% of the field capacity Pots were covered with aluminum foil and held for 3 days in a growth room at

28-30 °C and 14 hours of light. *Meloidogyne incognita* race 1 J2 were suspended in 10 ml of water and dispensed equally into each of five holes 5-cm deep in the soil of each pot. Five days later, one 5- to 10-cm-tall tobacco seedling was transplanted into each pot. A 20-20-20 (N-P-K) fertilizer was applied once per week, and insects were controlled as needed using recommended insecticides.

The two experiments were run for 45 days and 46 days, respectively. Plant height and fresh shoot weights were recorded. The root systems were washed free of soil, excess water was removed with paper towels, and the roots were weighed and then stored in plastic bags at 4 °C. The following day, roots were stained with Phloxine B (Dickson and Struhel, 1965), and the numbers of root galls and egg masses were counted. Eggs were extracted with 1.05% sodium hypochlorite (Hussey, 1971) and the number of eggs counted.

Nematode Origin

The *Meloidogyne incognita* race 1 isolate used in this study originated from tobacco grown on the University of Florida Green Acres Agronomy Research Farm, Alachua County, Florida. Species and race of the nematode population were identified by optical examination of the perineal pattern with the aid of light microscopy, and by the results of a differential host test (Hartman and Sasser, 1985). The nematode was cultured in a greenhouse on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) from a single egg mass. Eggs of *M. incognita* were extracted from infected tomato roots (Hussey and Barker, 1973), hatched on a Baermann (Rodríguez-Kábana and Pope, 1981), and used as 1- to 4-day-old J2 in the bioassay and the suppressive soil experiments.

Statistical Analysis

All data were subjected to factorial analysis of variance (ANOVA). Different means were separated and compared with Duncan multiple-range test or Student s *t*-test.

Results

Laboratory Experiments

Survival of the fungal populations and *P. penetrans* in the field soil varied with soil treatments (Table 5-1). Populations of fungi in autoclaved, microwaved, and air dried soil were different ($P \leq 0.05$); however, quantitative survival of fungi in the air-dried soil did not differ from the untreated soil ($P \leq 0.05$). The numbers of *P. penetrans* endospores per juvenile did not differ among the microwaved, air dried, and untreated soil ($P \leq 0.001$), but all endospores were killed in the autoclaved soil (Table 5-1). In the microwaved soil fungal populations were reduced in comparison to untreated or air dried soil, whereas the attachment of endospores was not affected.

Greenhouse Experiment

Suppressive soil treatment results in both experiment 1 and 2 were similar (Table 5-2). Interactions were detected for cultivars, soil treatments, and inoculation levels ($P \leq 0.05$); thus, data of the different cultivars are presented separately (Tables 5-3 and 5-4).

TABLE 5-1 Survival of soil fungi and *Pasteuria penetrans* in untreated soil or soil autoclaved twice for 1 5 hours at 55 kPa microwaved for 3 minutes/kg of soil air-dried for 2 weeks in the greenhouse

Soil treatments	Cfu of fungi per g of soil[a]	Mean number of *P. penetrans* endospores attached per 20 J2[b]
Autoclaved	0 c	0 b
Microwaved	2 x 10^3 b	10 a
Air-dried	2 7 x 10^4 a	13 a
Untreated	2,4 x 10^3 a	8 a

Values are means two experiments six replicates each Means within columns followed by the same letter do not differ at $P \leq 0$ 05 according to Duncan s multiple range test.

[a] The aumber af fungal propagules was determined by dilution platmg and is presented as colony forming units (cfu)

[b] The endospores of *P penetrans* were bioassayed on second-stage juveniles (J2) of *Meloidogyne incognita* race 1

TABLE 5-2 ANOVA table for the effects of tobacco cultivars, soil treatments (autoclaving, microwaving, air drying, and untreated) and *Meloidogyne incognita* race 1 inoculum levels (0 or 2 000 second stage juveniles) on nematode reproduction and plant performance

ANOVA	Number of galls per root system	Number of egg masses per root system	Number of eggs per root system	Total plant fresh weight	Shoot fresh weight	Root fresh weight	Total plant growth
			Experiment 1				
Cultivar (C)	***	***	***	**	**	**	**
Soil treatment (ST)	***	***	***	***	***	**	***
Inoculation level (I)	***	***	***	NS	NS	NS	NS
C x ST	***	***	***	***	**	***	**
C x I	***	***	***	NS	NS	NS	NS
I x ST	***	***	***	*	**	NS	**
I x ST x C	***	***	***	NS	*	NS	**
			Experiment 2				
Cultivar (C)	***	***	***	**	*	**	NS
Soil treatment (ST)	***	***	***	***	***	***	***
Inoculation level (I)	***	***	***	NS	NS	NS	NS
C x ST	**	***	***	***	**	***	**
C x I	***	***	***	NS	NS	NS	NS
I x ST	***	***	***	NS	**	NS	**
I x ST x C	***	***	***	NS	NS	NS	**

*, **, *** represent $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$ respectively, and NS = no differences at $P \leq 0.05$ according to Duncan's multiple-range test

TABLE 5.3 Effect of autoclaving, microwaving and air-drying on soil suppressiveness to *Meloidogyne* spp. and the expression of root knot on tobacco cultivar Coker 371-Gold (susceptible to both *Meloidogyne incognita* and *M. javanica*) following inoculation with 0 or 2 000 second stage juveniles (J2) of *M. incognita* race 1

Soil treatment	Nematode reproduction						Plant performance							
	Number of root galls per root system		Number of egg masses per root system		Number of eggs per root system		Total fresh weight (g)		Shoot weight (g)		Root weight (g)		Shoot height gained (cm)	
						Experiment 1								
	+[a]		+		+		+		+		+		+	
Autoclaved	223 a	0 b	191 a	0 b	13 086 a	0 c	89 a	98 a	77 a	82 a	13 a	17 a	27 a	26 a
Microwaved	129 b	0 b	25 b	0 b	1,312 b	0 c	79 a	72 b	61 a	56 c	17 a	15 a	18 a	16 b
Air dried	118 b	15 b	12 b	3 b	524 c	279 b	75 a	88 ab	60 a	72 ab	15 a	16 a	23 a	20 b
Untreated	107 b	102 a	35 b	21 a	1,290 b	828 a	74 a	75 b	62 a	58 b	12 a	17 a	22 a	19 b
						Experiment 2								
Autoclaved	470 a	0 c	437 a	0 b	49 219 a	0 c	52 a	74 a	37 a	61 a	14 a	14 a	4 a	7 a
Microwaved	105 b	0 c	74 b	0 b	1 858 b	0 c	54 a	54 b	43 a	41 b	11 a	12 a	5 a	3 b
Air dried	155 b	135 b	94 b	92 a	9 169 b	3 992 b	46 a	36 c	32 a	25 c	13 a	11 a	3 a	2 b
Untreated	283 b	229 a	99 b	117 a	6,051 b	13 582 a	51 a	58 b	36 a	44 b	13 a	14 a	3 a	4 a

Values are means of five replicates. Means within columns and within experiments followed by the same letter do not differ at $P \leq 0.05$ according to Duncan's multiple range test.

[a] + = Inoculated with 2 000 J2 of *M. incognita* race 1. − = No nematodes added (natural or resident population following air drying for 2 weeks in the greenhouse).

TABLE 5-4 Effect of autoclaving, microwaving and air drying on soil suppressiveness to *Meloidogyne* spp and the expression of root knot on tobacco cultivar Narthrup King 328 (resistant to *Meloidogyne incognita* but susceptible to *M javanica*) following inoculation with 0 or 2,000 second-stage juveniles (J2) of *M incognita* race 1

Soil treatment	Nematode reproduction						Plant performance					
	Number of root galls per root system		Number of egg masses per root system		Number of eggs per root system		Total fresh weight (g)		Shoot weight (g)		Root weight (g)		Shoot height gained (cm)	
	+		+		+		+		+		+		+	
	Experiment 1													
Autoclaved	1 2 b	0 b	1 b	0 b	118 b	0 b	127 a	102 a	104 a	84 a	23 a	21 a	26 a	17 a
Microwaved	0 3 b	0 b	0 b	0 b	0 b	0 b	92 b	74 b	71 b	57 b	21 a	18 a	14 b	13 b
Air dried	1 7 b	1 b	2 b	1 b	113 b	159 b	88 b	62 b	69 b	50 b	19 a	11 b	13 b	8 a
Untreated	15 a	14 a	6 a	10 a	385 a	395 a	74 b	94 a	59 b	73 a	15 a	21 a	13 b	16 ab
	Experiment 2													
Autoclaved	0 c	0 b	0 b	0 b	36 b	0 b	70 b	69 a	52 bc	55 a	18 a	15 b	4a	4 a
Microwaved	0 c	0 b	0 b	0 b	0 b	0 b	74 a	76 a	54 a	53 a	20 a	23 a	4 a	4 a
Air dried	60 eb	24 b	44 a	10 b	2,288 a	681 b	51 b	48 b	37 bc	38 b	15 a	12 b	3 a	3 a
Untreated	73 a	103 a	48 a	75 a	2,470 a	2,522 a	44 c	43 b	33 c	32 b	11 a	11 b	3 a	3 a

Values are means of five replicates Means within columns and within experiments followed by the same letter do not differ at $P \leq 0.05$ according to Duncan's multiple range test

* + = Inoculated with 2,000 J2 of *M incognita* race 1 = No nematodes added (natural or resident population following air drying for 2 weeks in the greenhouse)

When the susceptible cultivar received nematode inoculum, root galling, number of egg masses and individual eggs were less in microwaved, air-dried, and in untreated soil than in autoclaved soil ($P \leq 0.05$) (Table 5-3). In experiment 1, the number of eggs was further suppressed in the air-dried soil compared with other treatments ($P \leq 0.05$). However, this effect was not observed in experiment 2 in which the microwave treated soil with few fungi present (Table 5-1), was just as suppressive as the air-dried and untreated soil (Table 5-3). In comparison with the uninoculated treatments, nematode reproduction on susceptible Coker 371-Gold was lower in the autoclaved, microwaved, and air-dried soil, but not in the untreated soil ($P \leq 0.05$).

Meloidogyne incognita race 1 reproduced slightly on the resistant tobacco cultivar (Table 5-4). With the exception of the air-drying treatment in experiment 2, nematode reproduction on resistant Northrop King K-326 did not differ between inoculation levels ($P \leq 0.05$). When no nematode inoculum was added and generally when inoculum was added, root-knot damage in the untreated soils was higher than in the other treatments ($P \leq 0.05$).

Plant performance did not differ among treatments in the case of the inoculated susceptible tobacco cultivar (Table 5-3). However, when no inoculum was added, the total weight, shoot weight, and shoot height but not root weight, were generally greater in the autoclaved soil than that in the other treatments ($P \leq 0.05$). In experiment 1, the performance of the uninoculated resistant cultivar was generally favored in autoclaved and untreated soils (Table 5-4). The weight of inoculated roots of either cultivar, and of uninoculated roots of the susceptible cultivar was not affected by soil treatments (Tables 5-3 and 5-4).

Discussion

Nematode reproduction was suppressed consistently in the presence of viable *P penetrans* endospores The reduction most likely resulted from the infection of *M incognita* by *P penetrans*, since the numbers of egg masses and eggs were reduced more than the number of root galls. This supports earlier observations that infection by *P penetrans* causes sterility or reduced fecundity in females (Mankau, 1980a, Minton and Sayre 1989) Although nematode suppression by soilborne fungi and *P penetrans* could not be evaluated separately, an additive nematode suppression would be expected to cause differences in nematode reproduction between the microwaved and air-dried soils In one experimeot a significantly lower number of eggs was observed in air dried soil However, since this effect could not be reproduced in experiment 2, the roles of fuogi in nematode suppression could not be elucidated These results support the hypothesis that *P penetrans* is the main contributor to the suppressiveness of the soil

The reproduction of *Meloidogyne incognita* race 1 on the resistant cultivar is not unusual in that it has beeo reported in several other studies with plants carrying the same Mi 1 resistance gene (Huang 1986 Schaeider, 1991, Veech and Endo 1970) Root-knot damage on the resistant cultivar in the untreated soil probably resulted from infection by the natural population *of M javanica* Nematode reproduction was not increased by additional nematode inoculum

The generally improved plant performance (except root weight) observed in autoclaved soil was probably induced by an increased release of outnents into the soil solution (Ferriss 1984) In experiment 2 inoculated plants did not benefit from the autoclave treatment. In this case water and nutrieot absorption may have been limited by the heavy nematode infection (Hussey, 1985) Severe stunting and yield reductions caused

by *M incognita* on resistant cultivars was previously described as being caused by the hypersensitive response of the resistant roots to the nematode infection (Barker et al , 1981)

The suppressive nature of soils containing *P penetrans* has been reported previously (Bird and Brisbane, 1988, Dickson et al., 1994 Mankau 1980a) In all cited cases continuous replanting of a nematode-susceptible crop apparently caused the nematode antagonist to increase to suppressive levels. Bird and Brisbane (1988) demonstrated qualitatively the biological origin of nematode suppression caused by *P penetrans* in field soil In the present study, the lower number of developing *Meloidogyne* spp in the microwaved soil with viable *P penetrans* endospores suggests that *P penetrans* was important in nematode suppression and the reduction of plant damage Further Improvement of the bioassay for nematophagous fungi is required in order to make conclusions about their contribution to the suppressiveness of a soil to plant-parasitic nematodes

CHAPTER 6
POPULATION DENSITY DEVELOPMENT OF *MELOIDOGYNE ARENARIA* AND *PASTEURIA PENETRANS* IN METHYL BROMIDE TREATED SOIL

Introduction

The annual yield suppression of the world's major crops caused by plant parasitic nematodes was estimated to be approximately 12% (Sasser and Freckman, 1987). The increasing limitation in number and applicability of registered nematicides has stimulated increased research on biological alternatives. Among many naturally occurring organisms antagonistic to root-knot nematodes *Pasteuria penetrans* has the potential to contribute to natural and induced nematode control (Bird and Brisbane, 1988; Brown et al., 1985; Channer and Gowen, 1988; Dickson et al., 1994; Kerry, 1990; Mankau, 1980; Minton and Sayre, 1989; Oostendorp et al., 1991a; Sayre and Starr, 1988; Stirling and White, 1982). Unfortunately this obligate bacterial parasite cannot be mass-reared *in vitro*, which limits economic application to potting soil and small plot agriculture. Until a procedure of mass cultivation is developed, and *P. penetrans* is successfully commercialized as a biological control agent, the emphasis has to be on amplification of the pathogen in soil.

Levels of *P. penetrans* that are suppressive to populations of *Meloidogyne arenaria* can be inoculated into root-knot nematode infested soil (Chen et al., 1996b, 1997c) or can built up in soil over time in the presence of a nematode host and a susceptible crop (Dickson et al., 1994; Minton and Sayre, 1989; Oostendorp et al., 1991a, Chapters 2 and 4). Nematode suppressive levels in peanut (*Arachis hypogaea* L.) took 3 years of cropping

to develop, with crop losses in the first 2 years of a microplot study (Oosteodorp et al., 1991a, Chapter 2)

Increasing the suppressiveness of a soil to disease on a susceptible host with the simultaneous cultivation of a resistant or tolerant crop in an intercropping system with the susceptible crop may limit economic losses Intercropping is defined as growing two or more crops simultaneously on the same land (Lewandowski 1987) This cropping technique is practiced frequently in developing countries but is rarely used in developed countries like the United States (Francis and Decoteau 1993) Intercropping can reduce management input (Lewandowski 1987) and increase yields especially when cereals are intercropped with nitrogen fixing legumes (Elmore and Jacobs 1986) The cropping of sweet corn (*Zea mays* L.) and pole bean (*Phaseolus vulgaris* L.) together simultaneously would be a possible example of such an intercropping system The growth of sweet corn has been shown to be unaffected by a bean intercrop (Willey and Osiru, 1972) and many cultivars of sweet corn can support high population densities of root-knot nematodes without suffering yield reductions (McSorley personal communication) Bean is generally susceptible to root knot nematodes (Sikora and Greco 1990) therefore this crop can be used to amplify the nematode and its biological antagonist, *P penetrans*

The objectives of this experiment were to monitor the population development of *Meloidogyne arenaria* and *P penetrans* on a bean sweet corn intercrop in rotation with peanut, and to determine the population density of *P penetrans* that provided suppression of root knot nematodes.

Materials and Methods

Microplot experiment. At the University of Florida Green Acres Agronomy Farm, Alachua County, 90 microplots (each 76-cm in diameter) were arranged in 10 rows of nine plots with a distance of 1.5 m between plots in a loamy, siliceous, hyperthermic Grossarenic Paleudults with 90% sand, 4% silt, 6% clay, and 1.8% organic matter. In April of 1984, the microplots were fumigated with methyl bromide (98% methyl bromide plus 2% chloropicrin) at 977 kg/ha under 3 mm polyethylene mulch before inoculation with *M. javanica* (Neal) Chitwood and *Paecilomyces lilacinus* (Thom) Samson, and planted to crops supporting high levels of *Meloidogyne* spp. (Hewlett et al., 1988). In 1987, the plots were again treated with methyl bromide at 977 kg/ha under 3 mm polyethylene mulch and either inoculated with *M. arenaria* alone, with *M. arenaria* plus *P. penetrans*, or maintained free of nematodes and bacteria by periodic fumigation with methyl bromide. For the following 7 years, plots were planted to peanut during the summers, and maintained under rye, wheat or vetch, and bare fallow during the winters.

For the present study, all 90 microplots were fumigated with 977 kg/ha of methyl bromide as described above, on 10 March 1994. Thirty days later, the plots where uncovered. After aeration for 19 days, six 5-cm-deep holes and six 10-cm-deep holes (2.5-cm diameter) were evenly distributed throughout each plot. Inoculum of 10^6 eggs of *Meloidogyne arenaria* race 1 per microplot was applied uniformly in 2 liters of water over the soil. A potato rake was used to further incorporate the nematodes and leave the soil surface level. A 5-cm-deep furrow was made in a north-south direction through the middle of each plot. For intercropping system 1 (Fig. 6-1), sweet corn (*Zea mays* var. saccharata cv. Silver Queen) was planted on 28 April in the furrows. Two weeks later the corn plants were thinned to six plants per plot, and pole beans (*Phaseolus vulgaris* cv.

Fig. 6-1 Cut away illustration of a sweet corn (middle plant) and pole bean intercropping system in field microplots (76 cm diameter)

Kentucky Wonder) were planted to each side of the corn. After germination, the pole bean plants were thinned to a stand of three plants on each side of the corn. Plots were irrigated by a microjet sprinkler system at the rate of 2 ml/cm^2 per day. The plots were fertilized with ammonium sulfate (18-0-0) and potash (0-0-10) with a total of 385 kg N/ha and 320 kg K/ha respectively provided in one at planting and three post planting applications. Over a period of 1 week in mid July, plant shoots were cut off at ground level and removed from the microplots. Three root systems of both sweet corn and pole bean were harvested from each plot, and galling indices were recorded according to the scale: 0 = no galls, 1 = 1–10, 2 = 11–20, 3 = 21–55, 4 = 56–80, and 5 = 81–100% of roots galled (Barker et al. 1986). The root systems were returned to their original plots and turned into the soil. After 3 weeks of bare fallow, the soil in each plot was leveled and the same crops were planted in an east–west direction (intercropping system 2). Plots were maintained as described above. After 86 days, the root systems were harvested. Three 2.5-cm-diameter, 15-cm-deep soil cores were taken for nematode extraction with a cone-shaped auger from each plot. Nematodes were extracted from 100-cm^3 subsamples using centrifugal flotation (Jenkins 1964). The numbers of second-stage juveniles (J2), endospores per juvenile, and the rate of attachment by *P. penetrans* (as averages of 20 randomly selected individuals) were determined with the aid of an inverted microscope at ×400 magnification. For the winter plots were planted in mid-November to a cover crop of hairy vetch (*Vicia villosa* Roth). On 19 April 1995, the plant shoots of the winter cover crop were removed, and their fresh and dry weights were determined. Soil samples were collected, nematodes were extracted, and data were recorded as above.

On 2 May 1995, peanuts cv. Florunner were germinated on filter paper in petri dishes and six seeds were planted 5 days thereafter in each microplot. Two weeks later, the peanut plants were thinned to three plants per microplot (Fig. 6-2). Plots were irrigated as

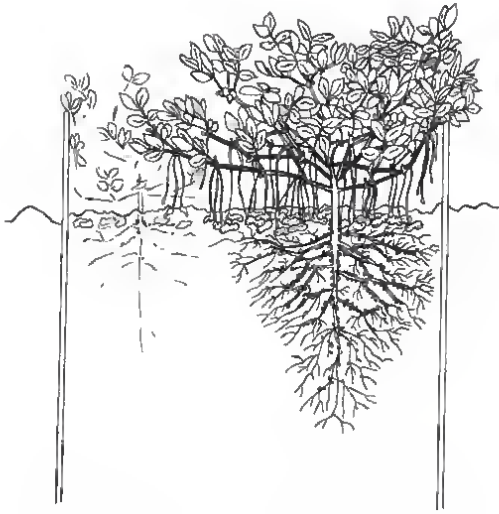

Fig 6-2 Cut away illustration of a peanut crop in field microplots (76 cm diameter)

needed using the system described above. After 123 days, peanut plants were lifted out of the ground and the galling indices were determined and recorded for each root system, and for pods and pegs. A five-core soil sample was taken with a cone-shaped auger from the center of each plot. Data on nematode and *P. penetrans* population densities were collected as described above. After 5 days, peanut pods were picked, dried at 60 °C until their moisture level was reduced to about 10% and weighed. Insect and disease control were maintained based on recommendations for Florida (Whitty et al., 1975).

Laboratory bioassays. Throughout the two cropping seasons, the population density of *P. penetrans* was estimated by bioassay using J2 of *M. arenaria* race 1 as a host. Three, 2.5-cm-diameter, 15-cm-deep soil core samples were collected with a cone-shaped auger from each microplot on 21 April 1994, 21 April 1995, and 7 September 1995 (referred to as spring 1994, spring 1995, and fall 1995, respectively). The soil was screened through a sieve with 0.8 mm openings, and the moisture content was determined by drying 100 g of soil at 60 °C until weight was consistent. The soil moisture content was 6.2%, 6.5%, and 5.7% in the spring of 1994, spring of 1995, and fall of 1995, respectively. A 50-g sample of soil from each microplot was placed in a petri dish. The soil then was subjected to microwave treatment equivalent to 4 minutes/kg of soil at full power in the 18-liter cavity of a microwave oven (1,500 Watts, 2,450 MHz) (Tappan Appliance, Mansfield, OH), which caused the temperature to rise to about 75 °C. About 500, 1- to 4-day-old J2 were suspended in 10 ml of water and applied uniformly to the soil using a pipette. Petri plates were incubated at room temperature for 48 hours and stored at 4 °C until further processed. Within the following 2 days, J2 were extracted by centrifugal flotation (Jenkins, 1964). The number of endospores per juvenile and the rate of attachment by *P. penetrans* (as averages of 20 randomly selected individuals) were determined with the aid of an inverted microscope at ×400 magnification. In the summer of 1996, tomato seedlings were transplanted into 5-cm-diameter plastic pots filled with 250-g samples of

soil from each microplot, which was microwave treated at the rate of 3 minutes/kg of soil After two days, 1,000 ± 46 1- to 4-day-old J2 of *M arenaria* race 1 were suspended in 5 ml of water and added to the pots Pots were maintained in the greenhouse for 600 degree days (18 °C base temperature), 20 female nematodes were dissected per root system and the presence of *P penetrans* endospores was determined with the aid of a microscope at ×1000 magnification.

Nematode inoculum. *Meloidogyne arenaria* race 1 was produced on tomato *Lycopersicon esculentum* Mill cv Rutgers in a greenhouse For the microplot inoculation, nematode eggs were extracted with 1 05% sodium hypochlorite (Hussey 1971) from the infected root systems (Hussey, 1973) and stored for a maximum of 3 days at 15 °C. The egg suspension then was adjusted to 4,000 eggs/ml of water, and further diluted to 500 eggs/per ml of water, resulting in 2 liters of inoculum per microplot. To test the viability of eggs used for microplot inoculation 4,000 eggs were placed on a modified Baermann funnel and incubated at 22 °C. The hatching rates of the *M arenaria* race 1 were 5 5%, 18 5%, and 39% after 48, 72, and 120 hours, respectively For the bioassays nematode eggs were collected as described above and hatched on a modified Baermann funnel at 28 °C up to 4 days before inoculation

Statistical analysis. The density of nematodes and the numbers of endospores per juvenile were transformed with $\log_{10}(x+1)$ before analysis Data that were calculated as percentages were transformed with arcsin ($\sqrt{x}$) before analysis Plots were ranked and divided into 10 groups of 9 microplots each based on the number of endospores per juvenile of *M arenaria* race 1 as determined by a bioassay in the spring of 1994 The change over time was determined by subtraction of the first or second data set from the final data set. The grouped data were subjected to analysis of variance (ANOVA), and group means were compared with Duncan's multiple range test Spearman correlation coefficients (Sokal and Rohlf 1969) were performed on the ranks of the ungrouped data of

all 90 microplots. Linear regressions were performed to determine the relationship between the initial *P. penetrans* density and the final nematode and endospore population densities. The linear regressions of the changes in population densities between spring and fall data of 1995 on the initial *P. penetrans* density were used to estimate the rate at which these changes were influenced by the initial endospore density.

Results

Bioassay experiments. Grouping of the ranked 90 microplots resulted in 10 groups with mean numbers of 0, 0, 1, 3, 4.5, 7, 9, 12, and 18 endospores attached per juvenile (Table 6-1). In the spring of 1994, the mean numbers of endospores per juvenile for groups 2 to 10 were different ($P \leq 0.001$) (Tables 6-2 and 6-3), which was expected since the groups were formed by ranked data. In the following spring, the numbers of attached endospores generally decreased, especially in plots that previously yielded the highest numbers of endospores per juvenile. In the final bioassay of fall 1995, the estimated *P. penetrans* population density increased in all groups except group number 1, resulting in three different density levels ($P \leq 0.05$) with ranges of means of 1-6 endospores/juvenile, 23 endospores/juvenile, and 53-68 endospores/juvenile. Two groups with mean endospore counts of 45 an 40 did not differ from the intermediate group and the high group.

Over two seasons, an increase between 1.1 and 5.1 endospores/juvenile for groups 1 to 3 was less than the increase between 35.1 and 55.8 endospores/juvenile in groups 5 to 10 ($P \leq 0.05$) (Table 6-3). With every endospore attached per juvenile in the spring of 1994, an average addition of 3.7 endospores/juvenile was observed over the two seasons ($Y = 2.4 + 3.7x$) (Fig. 6-3 A). Similarly, when the attachment rate in the spring of 1994 was compared to the difference in attachment between the spring and fall of 1995, the rate of change (slope) was 2.9, which indicates that with every endospore attached in the

TABLE 6-1 Grouping of 90 microplots based on the mean number of *Pasteuria penetrans* endospores attached per second stage juvenile of *Meloidogyne arenaria* race 1 in a bioassay on the microplot soil conducted in the spring of 1994

	Group									
Replicate	1	2	3	4	5	6	7	8	9	10
1	0	0	1	1.9	3.3	4.2	5.9	7.8	10.7	14
2	0	0	1	2	3.4	4.4	5.9	8	10.8	14.4
3	0	0	1	2	3.4	4.8	6.2	8	10.9	14.9
4	0	0	1	2.3	3.4	4.8	6.4	8.8	10.9	15.3
5	0	0	1	2.6	3.5	4.9	6.6	9	11.5	15.4
6	0	0	1	2.6	3.6	5	6.7	9	11.8	18.3
7	0	0	1	3	3.7	5.1	7	10.3	12	19.3
8	0	0	1.3	3	3.8	5.2	7	10.5	12.8	23.2
9	0	0	1.7	3.3	4.1	5.8	7.1	10.7	13.6	24.7
Group mean	0	0	1.1	2.5	3.6	4.9	6.5	9.1	11.7	17.5

Twenty randomly selected juveniles were observed per sample

TABLE 6-2 Main effect of group differences of groups with different *Pasteuria penetrans* endospore densities in microplots intercropped to two cycles of corn and beans in rotation with peanut on the population development of *P. penetrans* and *Meloidogyne arenaria* race 1 as determined by endospore attachment bioassay, soil sample extraction, and galling index.[a]

	Endospore attachment bioassay		Soil sample			Galling index		
	Endospores per juvenile	% Attachment	Juveniles per 100 cm³ of soil	Endospores per juvenile	% Attachment	Corn	Bean	Peanut
Spring 1994	***	***						
Summer 1994	-	-				***	**	
Fall 1994	-		*	***	***	**	**	
Spring 1995	***	***	NS	***	***			
Fall 1995	***	***	***	***	***			***
			Change over time					
1 Season	***	***	NS	NS	NS	NS	NS	
2 Seasons	***	NS	***	***	***	-	-	***

[a] The comparisons were among 10 groups of nine microplots with mean numbers of 0, 0.1, 3, 4, 5, 7, 9, 12, and 18 attached endospores of *P. penetrans* per juvenile

*, **, *** represent $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively

NS = no significant differences at $P \leq 0.05$ according to the analysis of variance (ANOVA)

- represents no observation

TABLE 6-3 Mean number of *Pasteuria penetrans* endospores attached per second stage juvenile of *Meloidogyne arenaria* race 1 in bioassays of microplot soil with different initial endospore densities intercropped with two cycles of corn and beans in 1994 planted to hairy vetch in the winter of 1994-95, and cropped with peanut in the summer of 1995

	Spring 1994		Spring, 1995		Fall 1995		Change over time[b]	
Group[a]	Mean	s^2	Mean	s^2	Mean	s^2	1 Season	2 Seasons
1	0 i	0	1.0 bcd	0.02	1.1 c	0.81	0.1 h	1.1 b
2	0 i	0	0.4 d	0.12	1.2 e	2.03	0.8 h	1.2 b
3	1.1 h	0	0.4 d	0.17	6.2 e	82.1	5.6 g	5.1 b
4	2.5 g	0	0.8 cd	0.07	22.5 b	111	21.7 f	20.0 ab
5	3.6 f	0	1.2 bcd	0.04	45.3 ab	72.6	44.0 d	41.7 a
6	4.9 e	0	1.6 abc	0	40.0 ab	0.23	38.4 e	35.1 a
7	6.5 d	0	2.7 a	0.6	53.2 a	4.88	50.5 d	46.7 a
8	9.1 c	0	1.7 abc	0	56.0 a	3.89	54.3 c	46.9 a
9	11.7 b	0	2.2 ab	0.02	67.5 a	5.99	65.3 a	55.8 a
10	17.5 a	0.04	2.0 abc	0	63.2 a	16.2	61.2 b	45.7a

The data from the 90 bioassays were transformed with $\log_{10}(x + 1)$ and ranked based on spring 1994 data before being grouped and subjected to ANOVA.

Presented data are untransformed. Means within columns followed by the same letter are not different at $P \leq 0.05$ according to Duncan's multiple range test

[a] Each group is comprised of nine replicates. 20 randomly selected juveniles were observed per replicate

[b] The change over time was determined by subtraction of the mean number of endospores attached per juvenile from data attained in the spring of 1995 from the fall of 1995 for one season, and of the spring of 1994 from the fall of 1995 for two seasons

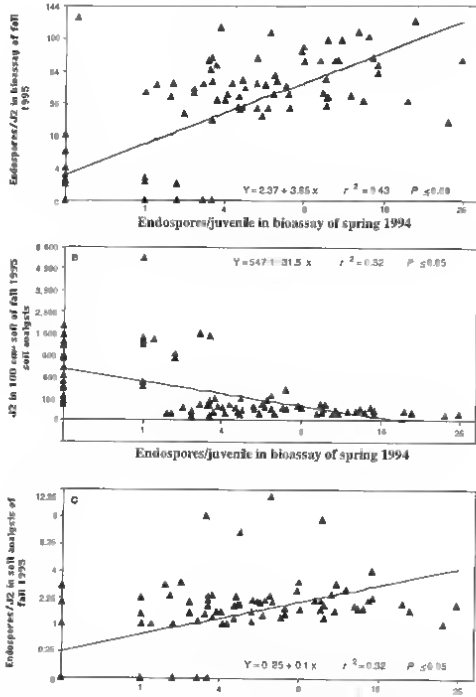
Endospores/J2 in bioassay of fall 1995
Endospores/juvenile in bioassay of spring 1994
Y = 2.37 + 3.85 x
r² = 0.43
P ≤0.05
B
J2 in 100 cm³ soil of fall 1995 soil analysis
Y = 547 - 31.5 x
r² = 0.32
P ≤0.05
C
Endospores/J2 in soil analysis of fall 1995
Y = 0.85 + 0.1 x
r² = 0.32
P ≤0.05

Fig 6-3 Relationship between the initial endospore population density (estimated by the number of endospores of *Pasteuria penetrans* per second stage juvenile of *Meloidogyne arenaria* in the spring 1994 bioassay) and A) the final number of endospores per juvenile as determined by bioassay B) the final number of second stage juvenile(s) (J2) per 100 cm^3 soil and C) the final number of endospores per juvenile as determined by soil sample extraction

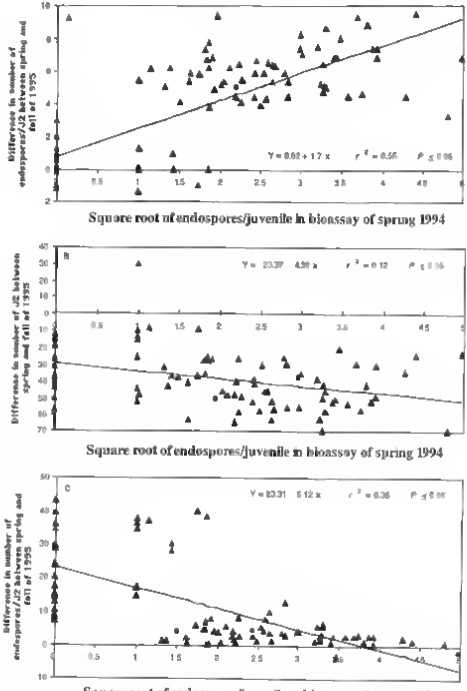
A
Difference in number of endospores/J2 between spring and fall of 1995
Y = 0.60 + 1.7 x
r² = 0.56
P ≤ 0.05
Square root of endospores/juvenile in bioassay of sprung 1994
B
Difference in number of J2 between spring and fall of 1995
Square root of endospores/juvenile in bioassay of spring 1994
C
Difference in number of endospores/J2 between spring and fall of 1995
Y = 83.31 - 6.12 x
r² = 0.36
P ≤ 0.05
Square root of endospores/juvenile in bioassay of sprung 1994

Fig. 6-4 Rate of change for A) the number of endospores of *Pasteuria penetrans* per juvenile of *Meloidogyne arenaria* as determined by bioassay B) the number of second stage juveniles (J2)/100 cm^3 soil and C) the number of endospores/juvenile as determined in soil sample extraction based on the initial endospore population density (estimated by the number of endospores per juvenile in the spring 1994 bioassay) and the change over the 1995 season

bioassay of spring 1994, the change in the final estimated population density increased over the 1995 season by 2.9 endospores ($Y = 0.67 + 2.9x$ or $Y = 0.82 + 1.7x$ after transformation with square root of x) (Fig. 6-4 A).

If compared with the numbers of endospores per juvenile, the trends observed for the percentages of J2 with attached endospores were similar (Table 6-4). In the spring of 1994, the attachment by *P. penetrans* increased through group 5, and leveled off at 87-99% in groups 5 to 10. In the spring of 1995, 29% and 24% of the J2 in groups 1 and 2 had endospores attached, whereas only healthy J2 were recovered from this group in the previous spring. The highest rates of attachment leveled off in groups 5 to 10 with 57-76% of the juveniles attached. In the final bioassay (Fall, 1995), an overall increase raised the attachment rates of the three lowest groups to 34-48%, the intermediate group four to 85%, and the six highest groups to 98-100% ($P \leq 0.05$). The three lowest groups had lesser increases in attachment over one season than the seven highest groups. However, no difference was observed when the change over both seasons was considered.

Microplot soil populations. Under the corn-bean intercropping systems in rotation with peanut, the *M. arenaria* densities and *P. penetrans* endospore densities, as well as the galling indices were affected by the pretreatment endospore densities in the microplot soil at each sampling date ($P \leq 0.05$, $P \leq 0.001$) (Table 6-2). No differences were noted in the nematode population densities in spring 1995, as well in the population developments of the nematode and the bacterium and change in galling indices over one season. Over the two seasons, the endospore attachment, nematode population densities and galling indices were affected by the different endospore densities in the microplots ($P \leq 0.001$).

During the first season of two consecutive corn and bean intercropping systems, the nematode population density increased in the fall of 1994 to an average (across all groups) of 1,835 J2/100 cm^3 of soil (Table 6-5). In the spring of 1995, the nematode

TABLE 6-4 Percentage of second-stage juveniles of *Meloidogyne arenaria* race 1 with endospores of *Pasteuria penetrans* attached in bioassays of microplot soil with different initial endospore densities intercropped with two cycles of corn and beans in 1994, planted to hairy vetch in the winter of 1994-95, and cropped with [illegible] in the summer of 1995

	Spring 1994		Spring 1995		Fall 1995		Change over time[b]	
Group[a]	Mean	s^2	Mean	s^2	Mean	s^2	1 Season	2 Seasons
1	0 e	0	29 c	2	34 c	13	5 b	34 a
2	0 e	0	24 c	9	48 c	26	24 b	48 a
3	33 d	2	17 c	4	48 c	30	31 b	15 a
4	69 c	22	37 bc	6	88 b	52	51 a	19 a
5	87 b	18	57 ab	8	98 ab	27	41 a	11 a
6	96 ab	6	62 a	4	100 a	0	38 a	4 a
7	99 a	2	67 a	10	100 a	0	33 a	1 a
8	99 a	2	76 a	10	100 a	1	24 a	1 a
9	99 a	4	72 a	6	100 a	0	28 a	1 a
10	99 a	3	63 a	2	100 a	0	37 a	1 a

Means are based on nine samples. 20 randomly selected juveniles were observed per sample. The data were transformed with arcsin ($\sqrt{x}$) before being grouped and subjected to ANOVA.

Presented data are untransformed. Means within columns followed by the same letter are not different at $P \leq 0.05$ according to Duncan's multiple range test.

[a] Each group is comprised of nine replicates which were ranked based on the number of endospores per juvenile in the bioassay of the spring of 1994.

[b] The change over time was determined by subtraction of the mean percentage of juveniles with attached endospores of *P. penetrans* from data attained in the spring of 1995 from the fall of 1995 for one season, and of the spring of 1994 from the fall of 1995 for two seasons.

TABLE 6.5 Number of second-stage juveniles of *Meloidogyne arenaria* race 1 per 100 cm^3 of microplot soil with different initial *Pasteuria penetrans* endospore densities intercropped with two cycles of corn and beans in 1994, planted to hairy vetch in the winter of 1994–95 and cropped with peanut in the summer of 1995 [a]

	Fall 1994		Spring, 1995		Fall 1995		Change over time [c]	
Group [b]	Mean	s^2	Mean	s^2	Mean	s^2	1 Season	2 Seasons
1	2,312 ab	6 806	2,692 a	22,348	513 ab	11 548	-2 179 abc	-1,799 a
2	2,425 ab	3 835	3.151 a	2 883	430 ab	24 547	-2,721 bcd	1995 a
3	2,627 a	33 865	2,426 a	28,659	945 a	189 493	-1 481 a	-1,682 a
4	1 446 b	9 123	2,583 a	20,488	194 bc	45,089	-2,389 bcd	-1,252 a
5	1 709 ab	26 808	1 726 a	23 860	91 c	15,401	-1 635 ab	-1 618 a
6	1.726 ab	33,605	2,642 a	24,652	24 c	33	-2,618 cd	-1.702 a
7	1 552 b	2 843	3 076 a	10,385	36 c	63	-3 040 d	-1 516 a
8	1,593 b	2,634	2 448 a	23 131	38 c	114	-2 410 bcd	-1,555 a
9	2 047 b	2,047	2,634 a	50 741	19 c	12	-2,615 cd	-2 028 a
10	1,544 b	18 117	2,124 a	65 022	12 c	2	-2 112 bcd	-1 532 a

Means are based on nine samples. The data were transformed with $\log_{10}(x + 1)$ before being grouped and subjected to ANOVA.

Presented data are untransformed. Means within columns followed by the same letter are not different at $P \leq 0.05$ according to Duncan's multiple-range test.

[a] In the spring of 1994, each microplot was inoculated with 10^5 eggs of *M. arenaria* race 1 (ca. 180 eggs/100 cm^3 of soil).

[b] Each group is comprised of nine replicates, which were ranked based on the number of endospores per juvenile in the bioassay of the spring of 1994.

[c] The change over time was determined by subtraction of the mean numbers of juveniles per 100 cm^3 of microplot soil attained in the spring of 1995 from the fall of 1995 for one season, and the fall of 1994 from the fall of 1995 for two seasons.

population density further increased to an average of 2,750 J2/100 cm^3 of soil and did not differ among the groups ($P \leq 0.05$). In the fall of 1995, the nematode population density declined drastically, ranging from the low levels of 12 to 194 J2/100 cm^3 of soil in groups 4 to 10, to the high levels of 430-945 J2/100 cm^3 of soil in groups 1-3. The nematode reduction over one season in groups 6, 7, and 9 was greater than in groups 1 and 5 ($P \leq 0.05$). There were no differences in the change over two seasons. With every endospore attached per juvenile in the spring 1994 bioassay, a decrease of 31.5 J2/100 cm^3 of soil was observed over the two seasons ($Y = 547.1 - 31.5x$) (Fig. 6-3 B). Similarly, when the attachment rate in the spring of 1994 was compared to the difference in number of J2 between spring and fall of 1995, the rate of change (slope) was -19.3, which indicates that with every additional endospore attached in the bioassay of spring 1994, the suppression in the nematode population density over the 1995 season increased by 19.3 J2 ($Y = -546.2 - 19.3x$ or $Y = -23.4 - 4.4x$ after transformation with square root of x) (Fig. 6-4 B).

In the fall of 1994, the estimated number of endospores per juvenile in the microplot soil ranged from the lowest level of 0-1 in groups 1 to 4 to the highest level of 2.6 in group 10 ($P \leq 0.05$) (Table 6-6). Over one season, the change in the number of endospores was not different among groups, and in the final sample only group 10, with the highest attachment, showed a greater decrease in the number of endospores than the other nine groups. With every additional endospore attached per juvenile in the bioassay from the spring of 1994, an increase of 0.1 endospores/juvenile was observed in the soil analysis over the two seasons ($Y = 0.3 + 0.1x$) (Fig. 6-3 C). However, when the attachment rate in the spring of 1994 was compared to the difference in attachment between spring and fall of 1995, the rate of change was -37.5, which indicates that with every endospore attached in the bioassay from the spring of 1994, the decrease in the final estimated endospore population density in the soil increased over the 1995 season by 37.5

TABLE 6-6. Number of *Pasteuria penetrans* endospores attached per second stage juvenile of *Meloidogyne arenaria* race 1 in microplot soil with different initial endospore densities intercropped with two cycles of corn and beans in 1994, planted to hairy vetch in the winter of 1994-95, and cropped with peanut in the summer of 1995.

	Fall 1994		Spring 1995		Fall 1995		Change over time[b]	
Group[a]	Mean	s^2	Mean	s^2	Mean	s^2	1 Season	2 Seasons
1	0 d	0.01	0 c	0.05	0.1 c	0.04	0.1 a	0.1 a
2	0.1 cd	0.25	0 c	0.02	0.2 bc	0.26	0.2 a	0.1 a
3	0.1 cd	0.07	0 c	0.04	0.5 bc	0.27	0.5 a	0.4 a
4	1 bcd	0.14	0.6 bc	0.1	1.1 ab	0.16	0.7 a	0.1 a
5	1.6 bc	0.07	2.3 a	0.29	1.9 a	0.35	0.4 a	0.3 a
6	2.3 b	0.28	2 ab	0.01	1.9 a	0.08	-0.1 a	-0.4 a
7	2.3 b	0.01	1.9 ab	0	2.6 a	0.2	0.7 a	0.3 a
8	2.8 b	0.01	2.4 a	0.01	1.9 a	0	-0.5 a	-0.9 a
9	3.1 b	0.29	3.4 a	7.83	2.5 a	0.08	-0.8 a	-0.5 a
10	8.6 a	40.2	4.1 a	1.26	2 a	0	-2.1 a	6.6 b

Means are based on nine soil samples. 20 randomly selected juveniles were observed per soil sample. The data were transformed with $\log_{10}(x + 1)$ before being grouped and subjected to ANOVA.

Presented data is untransformed. Means within columns followed by the same letter are not different at $P \leq 0.05$ according to Duncan's multiple range test.

[a] Each group is comprised of nine replicates which were ranked based on the number of endospores per juvenile in the bioassay of the spring of 1994.

[b] The change over time was determined by subtraction of the mean number of endospores attached per juvenile attained in the spring of 1995 from the fall of 1995 for 1 season, and of the fall of 1994 from the fall of 1995 for two seasons.

endospores/juvenile ($Y = 543\ 4 - 37\ 5x$ or $Y = 23\ 31 - 6\ 12x$ after transformation with square root of x) (Fig 6-4 C)

Soil samples taken in the fall of 1994 revealed attachment rates ranging from 3-10% at the lowest levels in groups 1 to 3 and 62-89% at the highest levels in groups 4 to 10 ($P \leq 0\ 05$) (Table 6-7) The attachment rates were similar throughout the remainder of the experiment However, the change over two seasons was different between groups 1, 2 and 3, for which attachment increased by 2-15%, and group 10 which had a 18% decrease in the attachment of endospores to J2 ($P \leq 0\ 05$)

In both consecutive corn and bean intercropping systems of the 1994 season, a variable degree of root galling was observed on both plants (Table 6-8) Root galling was generally higher in groups 1, 2, and 3 than in the other groups ($P \leq 0\ 05$) Pods and pegs of the peanut plants were equally well protected from galling galling indices between 0 and 1.2 for groups 4 to 10 were lower than the indices of 2.8-3 1 recorded for groups 1 to 3. No differences were observed among galling indices between the two corn and bean intercrops Over two seasons of corn and bean and then peanut the galling in groups 6 to 10 was reduced by 2 1 to 2.6 units, which was higher than the reduction by 0.3 to 1 1 units of groups 1, 3, 4 and 5

The rank of the numbers of endospores per juvenile and the rates of attachment recorded from the bioassays were positively correlated to the rank of the same observations collected by soil sample extraction (Table 6-9) ($P \leq 0\ 05$) Thus the estimated endospore density determined by the bioassay was representative for the estimated density in the microplot soil However, the ranks of all endospore counts, as well as attachment rates were negatively correlated to the ranks of the nematode population densities and galling indices showing that the nematode population density was negatively correlated with the endospore density Only the ranks of the nematode population density of spring 1995

TABLE 6-7 Percentage of second-stage juveniles of *Meloidogyne arenaria* race 1 with endospores of *Pasteuria penetrans* attached in microplot soil with different initial endospore densities intercropped with two cycles of corn and beans in 1994, planted to hairy vetch in the winter of 1994-95, and cropped with peanut in the summer of 1995

	Fall 1994		Spring 1995		Fall, 1995		Change over time[b]	
Group[a]	Mean	s^2	Mean	s^2	Mean	s^2	1 Season	2 Seasons
1	3 e	6	3 e	15	7 c	5	4 a	4 abc
2	6 e	12	5 e	15	8 c	15	3 a	2 abc
3	10 e	3	18 bc	7	25 c	7	7 a	15 a
4	62 b	3	42 b	5	54 b	1	12 a	-8 abcd
5	76 ab	5	71 a	3	59 b	5	-12 a	-17 cd
6	75 ab	16	76 a	11	69 ab	8	-7 a	6 abcd
7	87 a	22	69 a	15	82 a	16	13 a	-5 abcd
8	86 a	3	86 a	14	80 a	7	-6 a	-6 abcd
9	89 a	2	84 a	2	80 a	1	-4 a	-9 bcd
10	88 a	1	78 a	1	70 ab	2	-8 a	18 d

Means are based on nine soil samples, 20 randomly selected juveniles were observed per soil sample. The data were transformed with arcsin ($\sqrt{x}$) before being grouped and subjected to ANOVA.

Presented data are untransformed. Means within columns followed by the same letter are not different at $P \leq 0.05$ according to Duncan's multiple-range test.

[a] Each group is comprised of nine replicates, which were ranked based on the number of endospores per juvenile in the bioassay of the spring of 1994.

[b] The change over time was determined by subtraction of the mean percentage of juveniles with endospores attached in the spring of 1995 from the fall of 1995 for one season, and of the fall of 1994 from the fall of 1995 for two seasons.

TABLE 6.8 Root galling rates of two intercroping systems with corn and beans rotated with peanut grown in microplots grouped by different *Pasteuria penetrans* endospore densities, and inoculated with *Meloidogyne arenaria* in the spring of 1994

	Summer 1994 (First intercrop)		Fall 1994 (Second intercrop)		Fall 1995 Peanut		Change over time[b]		2 Seasons (two crops of corn and bean
Group[a]	A	B	C	D	E	F	G	H	I
1	2.9 abc	3.3 abc	3.3 a	3.9 ab	2.2 a	2.8 a	0.4 a	0.6 a	1.1 ab
2	3.1 ab	4.5 a	3.1 ab	4.4 a	1.1 a	2.9 a	0 a	0.1 a	-1.8 bc
3	3.4 a	4.2 ab	3.5 a	4.0 ab	3.9 a	3.1 a	0.1 a	-0.2 a	-0.3 a
4	1.8 d	2.7 bc	2.6 abc	3.8 ab	4.4 b	1.2 b	0.8 a	1.1 a	-0.4 a
5	2.2 bcd	2.7 cb	2.1 bc	2.5 c	2.4 bc	0.5 b	0.1 a	0.2 a	-0.9 ab
6	1.6 d	2.4 c	1.9 c	2.4 c	0.1 c	0 b	0.3 a	0 a	-2.1 c
7	1.7 d	2.1 c	2.1 bc	3.0 bc	0.4 c	0.2 b	0.4 a	0.9 a	-2.3 c
8	1.7 d	1.9 c	2.6 abc	2.7 bc	0.2 c	0 b	0.9 a	0.8 a	-2.6 c
9	1.9 cd	1.9 c	2.0 c	2.9 bc	0.1 c	0 b	0.1 a	1.0 a	-2.4 c
10	1.6 d	1.7 c	2.2 bc	3.1 bc	0.1 c	0.4 b	0.6 a	1.4 a	-2.4 c

Means are based on three root systems per microplot nine microplots per group Means within columns followed by the same letter are not different at $P \leq 0.05$ according to Duncan's multiple-range test.

The galling index scale 0 = no galls, 1 = 1-10, 2 = 11-20, 3 = 21-55, 4 = 56-80, 5 = 81-100% of roots galled

[a]Each group is comprised of nine replicates, which were ranked over the number of endospores per juvenile in the bioassay of the spring of 1994

[b]The change over time for corn and bean was determined by subtraction of the summer of 1994 from the fall of 1994, and by subtraction of the mean of fall of 1994 ((corn + bean)/2) from the mean of fall of 1995 ((root + pods and pegs)/2) for two seasons

TABLE 6-9 Spearman correlation coefficients of ranked data obtained from bioassays and from the analysis of soil extracted from 90 microplots containing varying population densities of *Pasteuria penetrans* endospores, plots were intercropped with two cycles of corn and bean in 1994, planted to hairy vetch in the winter of 1994-95, and cropped with peanut in the summer of 1995

	Bioassay						Soil sample extraction									Root galling indices (0-5)			
	Endospores per juvenile			Attachment rates (%)			Endospores per juvenile			Attachment rates(%)			Juveniles per 100 cm³ soil			Corn	Bean	Peanut	
	s 94[a]	s 95	f 95	s 94	s 95	f 95	f 94	s 95	f 95	f 94	s 95	f 95	f 94	s 95	f 95	f 94	f 94	Roots f 95	Pods f 95
Column	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	1																		
2	0.58	1																	
3	0.7	0.55	1																
3	0.89	0.55	0.71	1															
5	0.66	0.75	0.61	0.66	1														
6	0.62	0.58	0.78	0.69	0.57	1													
7	0.78	0.59	0.7	0.78	0.53	0.71	1												
8	0.76	0.67	0.71	0.76	0.7	0.69	0.78	1											
9	0.61	0.47	0.63	0.63	0.58	0.55	0.57	0.6	1										
10	0.78	0.59	0.8	0.8	0.66	0.72	0.9	0.76	0.66	1									
11	0.74	0.66	0.72	0.77	0.76	0.69	0.75	0.86	0.62	0.75	1								
12	0.73	0.64	0.69	0.81	0.7	0.68	0.75	0.7	0.79	0.78	0.74	1							
13	-0.32	-0.3	-0.37	-0.67	0.28	-0.32	-0.48	-0.45	-0.21	-0.5	-0.38	-0.32	1						
14	-0.11	-0.19	-0.3	0.19	-0.2	-0.14	-0.21	-0.32	0.28	-0.27	-0.33	-0.18	0.26	1					
15	0.65	-0.42	-0.59	-0.66	-0.55	-0.65	-0.67	-0.65	-0.58	-0.68	-0.62	-0.66	0.34	0.27	1				
16	0.42	-0.36	-0.49	-0.45	-0.36	-0.38	-0.48	-0.45	-0.39	-0.49	-0.52	-0.41	0.32	0.22	0.4	1			
17	-0.35	-0.39	-0.25	-0.39	0.43	0.29	-0.42	-0.39	-0.19	-0.38	-0.52	-0.43	0.32	0.3	0.37	0.22	1		
18	-0.67	-0.62	-0.55	0.72	-0.63	-0.62	0.68	-0.7	-0.47	-0.66	-0.74	-0.65	0.38	0.22	0.62	0.36	0.44	1	
19	-0.71	-0.55	-0.58	-0.77	-0.67	-0.58	-0.64	-0.66	-0.55	-0.66	-0.71	-0.65	0.39	0.26	0.62	0.41	0.33	0.85	1

Spearman correlation coefficients correlate the ranks of the data series. Correlation coefficients of ≥ 0.24 are significant ($P \leq 0.05$).

[a] s = spring sampling, and f = fall sampling during 1994 and 1995, respectively.

showed a random relationship with those of the attachment rates and the endospore densities of the spring data of 1994, as determined by the bioassays. Thus, the initial endospore density had no effect on the nematode population of the following spring.

Discussion

The nematode population became well established under the corn and bean intercropping systems through 1994 and remained at equally high levels in all microplots under the susceptible winter cover crop. No conclusions can be drawn about the effect of the intercropping system on the population development of *P. penetrans* in the soil, since no alternative treatment was included.

During the microplot sampling in the spring of 1995, 14% to 97% of the J2 escaped attachment by *P. penetrans* endospores, leaving at least 343 J2 free of *P. penetrans*/100 cm^3 of soil for each group. This density of nematodes would be capable of causing 90% yield loss in peanut (Porter et al., 1984). However, nematode populations of the five groups with ≥ 4.9 endospores per juvenile and an attachment rate of $\geq 96\%$ in the standard bioassay showed a decline in the incidence of galling, and the peanut crop was nearly free of galls. In contrast to the two fall samples, the high numbers of J2 in the spring of 1995 were not related to endospore counts and attachment rates in the bioassays. Therefore, in winter of 1994-95, the nematode population did not seem to be affected by the *P. penetrans* population density in the soil. This observation contradicts the results of other scientists (Oostendorp et al., 1991a), in which, despite the high temperature requirement of *P. penetrans* (Hatz, et al., 1992., Serracin et al., 1997, Stirling, 1981), endospore attachment increased, and nematode populations decreased during the winter.

season (Oostendorp et al 1991a, Chapter 2) The *P penetrans* populatioo in the microplot soil may have been unable to reproduce on the predominant nematode host.

In this experimeot the endospore populatioo developmeot determined by bioassays was not consistent with the endospore population development determined by soil sample extraction. A 3 7- and a 0 1 fold increase in the number of endospores per juvenile was estimated with the aid of bioassays or soil sample extraction, respectively A possible explanatioo might be given by a populatioo shift to *Meloidogyne* spp other than *M arenaria* that occurred by cultivating the root knot nematode susceptible pole bean (Mullin et al., 1991) It is possible that *M javanica* re established in the microplots from residual populations of previously conducted experiments (Dickson personal communication) The *P penetrans* population present in the microplots attached rather poorly to *M javanica* (Oostendorp et al., 1990) This could explain the good endospore attachment to *M arenaria* in the bioassays the poor attachmeot rates observed in soil samples during the corn and bean intercrop (if *M javanica* predominated on bean) and the suppressioo of *Meloidogyne* spp other than *M arenaria* in the course of the following peanut crop. Unfortunately the specification of the nematode population was not determined. It is also possible that in noperturbated soil, eodospore attachment was affected by other soilborne microorganisms In related studies as these microorganisms were selectively eliminated or redoced by the microwave treatment (Cheo et al., 1995, Ferriss 1984 Weibelzahl Fulton et al., 1996, Chapter 3) the number of endospores per juvenile increased.

After 2 years, the soils of seven groups with an initial mean number of 2.5 and more endospores per juvenile and a rate of attachment of 69% or higher developed to highly snppressive soils in which 88% or more of J2 were encumbered with 23 5 or more endospores *Pasteuria penetrans* is knowo to suppress root knot nematodes by affecting juvenile mobility and reducing or inhibiting female fecundity (Stirling 1991) Mobility

and infectivity of J2 is reduced when between 7 and 50 endospores are attached (Brown and Smart, 1985, Davies et al., 1988, 1990, Sell and Hansen, 1987, Stirling, 1984), and juveniles are prevented from invading roots when they are encumbered with 25 to 30 endospores (Stirling, 1984, Stirling et al., 1990). Previous experiments have shown that root systems can be protected from juvenile invasion by high numbers of *P. penetrans* endospores in the soil (Chen et al., 1996b, 1997, Weibelzahl-Fulton et al., 1996, Chapter 3).

Endospores of *P. penetrans* are relatively resistant to heat, desiccation, nematicides and other adverse environmental conditions (Stirling, 1991). The effect of methyl bromide (98% methyl bromide plus 2% chloropicrin) on the survival of the bacterial endospores is unknown at this time. Although the fumigation of soil containing *P. penetrans* with methyl bromide had little or no effect on the attachment of endospores to the J2 in a greenhouse bioassay, *P. penetrans* did not complete its life cycle with the formation of mature endospores (personal observation). This observation is in agreement with Freitas (1997) who reported that chloropicrin alone or in combination with methyl bromide was highly detrimental to *P. penetrans* because endospore formation was inhibited. Hence, the observed increase in endospore attachment in the present study may have resulted from the continuous release and dispersion of endospores from decomposing root material rather than from an endospore build up following soil fumigation.

The nematode population was generally lower in groups with an initial number of 2.5 or more endospores of *P. penetrans* per juvenile. Since methyl bromide treated endospores of *P. penetrans* appeared to be unable to reproduce (Freitas, 1997), the nematode suppression observed in this experiment most likely resulted from the prevention of root invasion by endospore encumbered juveniles. Endospore attachment is not necessarily followed by nematode infection (De Silva and Gowen, 1994; Freitas,

1997). In this experiment, the host-parasite development was limited to cuticular attachment only, similar to several previous observations (Stirling, 1991). Future scientists must exercise caution and actually determine parasitism before making conclusions about the host-parasite relationship.

It can be concluded that microplots with a certain *P. penetrans* population density remained in the original population density group through the course of the 2 year experiment. This observation may have been influenced by the effect of methyl bromide on the survival of the endospores in the soil, nonetheless, these density classes remained remarkably intact.

CHAPTER 7
SUMMARY

Since environmental problems have been associated with the application of nematicides (Thomason 1986) their use has led to great controversy and increasing attention is being given to alternative management tactics such as cultural practices, plant resistance and biological control of nematodes with microbial agents such as *Pasteuria penetrans* (Thorne) Sayre & Starr. The potential of *P. penetrans* in combination with cultural practices to suppress *Meloidogyne arenaria* on peanut (*Arachis hypogaea* L.) and *Meloidogyne* spp. on tobacco (*Nicotiana tabacum* L.) was investigated in laboratory greenhouse, microplot, and field experiments. A suppressive-soil test was developed to determine the role of *P. penetrans* in suppressive soils.

In 1987, before the initiation of the present study in 1990, 90 microplots were either infested with *M. arenaria* or with *M. arenaria* and *P. penetrans* or maintained nematode free by periodic fumigation (Oostendorp, 1991a). Rye, vetch or wheat or bare fallow was maintained for winter cover. The population development of *P. penetrans* and *M. arenaria* was monitored for the last 3.5 years (1990-1993) of the 6.5 year study. In the fifth, sixth, and seventh cropping season, nematodes were nearly eliminated from plots with the initial *P. penetrans* inoculum. The peanut yields did not differ from those in fumigated control plots. By the end of the 7 year study, *P. penetrans* had spread to all plots containing nematodes which caused an increasing portion of the population of second-stage juveniles (J2) to become encumbered with endospores and, hence,

detrimentally affected The increase in the estimated number of endospores in the soil was enhanced under susceptible vetch or wheat cover crops

The effect of microwave treatment on soil fungi and bacterial endospores was determined using dilution plating and soil bioassay An increase in microwave radiation time and soil water content revealed an increasing detrimental effect on *P penetrans* and other soil organisms A treatment time of 3 minutes/kg of soil at field capacity of 7% soil water reduced fungal populations but left the attachment by *P penetrans* nearly unaffected This microwave treatment was helpful because it reduced fungi that might also be a factor in suppressive soils

In a suppressive soil test, microplot soil was autoclaved microwaved, or air dried and bioassayed in the greenhouse using *M arenaria* on peanut as a host for potential biological control organisms The suppressive component was inhibited by autoclaving soil and this treatment resulted in heavy galling and egg mass formation on the root system Microwaving or air-drying soil did not affect the suppressiveness of the soils inoculated or contaminated with *P penetrans* Endospores in both soils existed in densities at which infectivity of J2 was reduced and the root systems were protected from severe root-knot infection *Pasteuria penetrans* was identified as the main contributer to the suppressiveness of the microplot soils. The suppressive soil test was a useful tool in the determination of the suppressive component in the soil. However the effects of the microwave treatment on soil organisms fluctuated with different soil characteristics, and requires additional experimentation

A mixed population of root-knot nematodes was observed to infest a tobacco field from which *Pasteuria penetrans* and several species of potential nematophagous fungi were isolated (Chen et al. 1994) This field site was planted to two tobacco cultivars (Coker 371 which is susceptible to *M incognita M javanica* and *M arenaria*, and

Northrup King 326 which is resistant to *M. incognita* but susceptible to *M. javanica* and *M. arenaria*), followed by three autumn cover crops (hairy indigo, forage sorghum, and weeds). It was fertilized with two rates of inorganic nitrogen fertilizers (89 and 158 kg of ammonium nitrate/ha). The nematode suppression increased over 2 years, and *P. penetrans* was identified to be suppressive to the root-knot nematodes. The endospore build up in the soil was slightly favored by a cropping history with the susceptible tobacco cultivar. The preferred host for the two isolated *P. penetrans* populations appeared to be *M. incognita* over *M. javanica*. Inorganic nitrogen and winter cover crops had no consistent effects on the endospore build up in the soil.

When the tobacco field soil was subjected to a suppressive soil test using *M. incognita* and tomato as hosts for potential nematode antagonists, *P. penetrans* was identified as the main contributor to the suppressiveness of the microplot soils. However when the experiment was replicated, soilborne fungi contributed to the nematode suppression. Thus the role of fungi in the nematode suppression could not be elucidated.

Ninety microplots were ranked and grouped based on the number of endospores per juvenile of *M. arenaria* as determined by a bioassay at the beginning of the experiment. The population development of *Meloidogyne* spp. and *P. penetrans* was monitored over 2 years planted with two cycles of a corn and bean intercrop in rotation with peanut. The soil of groups with an initial mean number of 3 and more endospores per juvenile and a rate of attachment of 69% or higher developed highly suppressive soil in which 88% or more of J2 were encumbered with 23 or more endospores. In a greenhouse bioassay, *P. penetrans* was not able to complete its life after soil with endospores was treatment with methyl bromide (98% methyl bromide plus 2% chloropicrin).

The current studies revealed that the continuous cultivation of crops susceptible to root-knot nematode for 3 to 4 consecutive years increases levels of *P. penetrans*

endospores in the soil from relatively low to highly suppressive levels. In the microplot environment the suppressiveness was maintained in the absence of nematodes for a period of 3 years before the experiment was terminated. Efforts should be directed toward the amplification of endospores in nature and in artificial mass production systems.

LIST OF REFERENCES

Abrantes I M de O., and N Vovlas 1988 A note on parasitism of the phytonematodes *Meloidogyne* sp and *Heterodera fici* by *Pasteuria penetrans* Canadian Journal of Zoology 66 2852 2854

Adiko A and S R Gowen 1994 Comparison of two inoculation methods of root knot nematodes for the assessment of the biological potential of *Pasteuria penetrans* Afro-Asian Journal of Nematology 4 32 34

Ahmad R M K Abbas, M A Kahn M Inam ul-Haq, N Javed and S T Sahi 1994 Evaluation of different methods of application of *Pasteuria penetrans* for biological control of root-knot of tomato (*Meloidogyne incognita*) Pakistan Journal of Nematology 12 155 160

Ahmad R M T Awan N Javed M A Khan, and H U Kahn 1990 Effect of some ecological factors on *Pasteuria penetrans* spore attachment to *Meloidogyne javanica*. Pakistan Journal of Phytopathology 2 31-36

Ahmed, R. and S R. Gowen 1991 Studies on the infection of *Meloidogyne* spp. with isolates of *Pasteuria penetrans* Nematologica Mediterranea 19 229 233

Al-Hazim, A S , A. A M. Ibrahim, and A. T Abdul-Razig 1993 Evalnation of a nematode-encapsnlating fungi complex for control of *Meloidogyne javanica* on potato Pakistan Journal of Nematology 11 139 149

Allen W A 1941 *Aphelenchuides megadorus*, a new species of Tylenchoidea (Nematoda) Proceedings of the Helminthological Society of Washington 8 21-23

Altherr, E , and C D Deboutteville 1972 Nématodes interstitiels des eaux douces des Etats-Unis d Amérique (Etats de Washington du Colorado et du Massachusetts) récoltés par Cl. Delamareteville Annales de Spéléologie 27 683 760

Baker K. F , and R J Cook 1984 Biological control of plant pathogens Originally printed 1974 American Pathological Society St Paul MN San Francisco W H Freeman

Baker K F and W H Fuller 1969 Soil treatment by microwave energy to destroy plant pathogens Phytopathology 59 193-197

Barker K. R. 1985 Nematode extraction and bioassays Pp 19-35 *in* K R. Barker C. C Carter and J N Sasser eds An advanced treatise on *Meloidogyne* vol II Methodology Raleigh. North Carolina State University Graphics

Barker, K R 1993 Resistance/tolerance and related concepts/terminology in plant nematology Plant Disease 77 111-113

Barker, K. R., F. A Todd, W W Shane and L A. Nelson. 1981 Interrelationship of *Meloidogyne* species with flue-cured tobacco. Journal of Nematology 13 67-79

Barker, K R J L. Townshend G W Bird, I J Thomason, and D W Dickson 1986 Determining nematode population responses to control agents Pp 283-296 *in* K D Hickey ed Methods for evaluating pesticides for control of plant pathogens. St Paul MN APS Press

Barooti, S 1989 Distribution of *Pasteuria penetrans* a parasite of nematodes in Iran Iranian Journal of Plant Pathology 25 9-10

Barron, G L. 1992 Lignolytic and cellulolytic fungi as predators and parasites. Pp 311-326 *in* G C Carroll and D T Wicklow eds The fungal community its organization and role in the ecosystem New York Marcel Dekker

Baxter, R I and C D Blake 1969 Oxygen and hatch of eggs and migration of larvae of *Meloidogyne javanica* Annals of Applied Biology 63 191-203

Bennett, F D 1974 Criteria for determination of candidate hosts and for selection of biotic agents Pp 87-96 *in* F G Maxwell and F A Harris eds Proceedings of the Summer Institute on biological control of plant insect disease Jackson MS University Press of Mississippi

Bernard, E. C., and T. L Niblack 1982 Review of *Hoplolyius* S Jacob (Nematoda. Pratylenchidae) Nematologica 28 101-109

Bhattacharya, D and G Swarup 1988 *Pasteuria penetrans* a pathogen of the genus *Heterodera* its effect on nematode biology and control Indian Journal of Nematology 18 61-70

Birchfield, W and A A Antonopoulus. 1976 Scanning electron microscopic observations of *Dubosqua penetrans* parasitizing root-knot larvae Journal of Nematology 8 272-273

Bird, A. F 1986 The influence of the actinomycete *Pasteuria penetrans*, on the host parasite relationship of the plant-parasitic nematode, *Meloidogyne javanica* Parasitology 93 571 580

Bird, A F 1988 A technique for staining the endospores of *Pasteuria penetrans* Revue de Nématologie 11 364-365

Bird A. F., J Bong and A Bacic 1989 Factors affecting adhesion of micro-organisms to the surface of plant-parasitic nematodes Parasitology 98 155 164

Bird A F- and P G Brisbane 1988 The influence of *Pasteuria penetrans* in field soils on the reproduction of root knot nematodes Revue de Nematologie 11 75-81

Bird, A F P G Brisbane, S G McClure, and W L Kimber 1990 Studies on the properties of the spores of some populations of *Pasteuria penetrans* Journal of Invertebrate Pathology 55 169-178

Bishop A H and D J Ellar 1991 Attempts to culture *Pasteuria penetrans in vitro* Biological Science Technology 1 101-114

Brown, S M J L Kepner and G C Smart, Jr 1985 Increased crop yields following application of *Bacillus penetrans* to field plots infested with *Meloidogyne incognita* Soil Biology and Biochemistry 17 483-486

Brown S M and D Norlmeyer 1985 Synergistic reduction in root galling by *Meloidogyne javanica* with *Pasteuria penetrans* and nematicides Revue de Nématologie 8 285 286

Brown S M and G C Smart Jr 1984 Attachment of *Bacillus penetrans* to *Meloidogyne incognita* Nematropica 14 171 172

Brown, S M and G C Smart, Jr 1985 Root penetration by *Meloidogyne incognita* juveniles infected with *Bacillus penetrans* Journal of Nematology 17 123 126

Bunt, J A 1987 Mode of action of nematicides Pp 461-468 *in* J A Veech and D W Dickson eds Vistas on nematology A commemorandum of the twenty-fifth anniversary of the Society of Nematologists Hyattsville MD Society of Nematologists.

Cappuccino J C, and N Sherman 1986 Microbiology, a laboratory manual Menlo Park CA Benjamin/Cummings Publishing

Channer, A G and S R Gowen. 1988 Preliminary studies on the potential of *Pasteuria penetrans* to control *Meloidogyne* species Proceedings of Brighton Crop Conference Pest and Disease Surrey UK. The British Crop Protection Council

Channer A. G, and S R Gowen. 1992 Selection for increased host resistance and increased pathogen specificity in the *Meloidogyne Pasteuria penetrans* interaction Fundamental and Applied Nematology 15 331-339

Charnecki J H 1997 *Pasteuria penetrans* spore proteins Potential function in attachment to *Meloidogyne* spp M S thesis University of Florida Gainesville.

Charnecki J H, J F Preston, and D W Dickson 1996 Fluorescent labeled endospores of *Pasteuria penetrans* for the characterization of the proteins involved in attachment to root knot nematodes Abstracts of the 96th general meeting of the society of microbiology Washington DC American Society of Microbiology (Abstr)

Chen S J Charnecki J F Preston, D W Dickson and J D Rice. 1997a Antibodies from chicken eggs as probe for antigens from *Pasteuria penetrans* endospores Journal of Nematology 29 268 275

Chen S , D W Dickson and D J Mitchell 1995 Effect of soil treatment on the survival of soil microorganisms Journal of Nematology 27 661-663

Chen S D W Dickson and E B Whitty 1994 Response of *Meloidogyne* spp to *Pasteuria penetrans* fungi, and cultural practices in tobacco Supplement to the Journal of Nematology 26 620-625

Chen Z X 1996 Biological control potential of *Pasteuria penetrans* Ph D dissertation University of Florida, Gainesville.

Chen, Z X and D W Dickson 1997 Estimating incidence of attachment of *Pasteuria penetrans* endospores to *Meloidogyne* spp with tally threshold. Journal of Nematology 29 289-295

Chen, Z. X., D W Dickson, L G Freitas and J F Preston 1997b Ultrastructure, morphology and sporogenesis of *Pasteuria penetrans* Phytopathology 87 273-283

Chen, Z X., D W Dickson, and T E Hewlett 1996a Quantification of endospore concentration of *Pasteuria penetrans* in tomato root material Journal of Nematology 28 50-55

Chen Z. X., D W Dickson R McSorley D J Mitchell and T E Hewlett 1996b Suppression of *Meloidogyne arenaria* race 1 by soil applications of *Pasteuria penetrans* Journal of Nematology 28 159-168

Chen Z X D W Dickson D J Mitchell R McSorley, and T E Hewlett. 1997a Suppression mechanisms of *Meloidogyne arenaria* race 1 by *Pasteuria penetrans* Journal of Nematology 29 1-8

Cho, M R., D W Dickson and T E Hewlett 1997 Comparison of inoculation methods and different host plants for production of *Pasteuria*. Journal of Nematology (in press) (Abstr)

Ciancio A 1995 Density dependent parasitism of *Xiphinema diversicaudatum* by *Pasteuria penetrans* in a naturally infested field. Phytopathology 85 144-149

Ciancio A R. Bonsignore N Volvas and F Lamberti 1994 Host record and spore morphometrics of *Pasteuria penetrans* group parasites of nematodes Journal of Invertebrate Pathology 63 260-267

Ciancio A., and M Bourjate. 1995 Relationship between *Pasteuria penetrans* infection levels and density of *Meloidogyne javanica* Nematologia Mediterranea 23 43-49

Ciancio A., S Landriscina, and L Serano. 1987 Indagini sulla nematofauna degli agrumi nell Italia Meridionale. Informatore Fitopatologico 37 55-57

Ciancio A and R Mankau 1989a. Note on *Pasteuria* sp parasitic to longidorid nematodes Nematropica 19 105-109

Ciancio A. and R Mankau 1989b The occurrence of *Pasteuria penetrans* like parasites in several Cephalobidae species. Journal of Nematology 21 556 (Abstr)

Ciancio, A., R Mankau, and M. Mundo-Ocampo. 1992 Parasitism of *Helicotylenchus lobus* by *Pasteuria penetrans* in naturally infested soil Journal of Nematology 24 29-35

Cobb, N A. 1906 *Fungus maladies* of the sugar cane with notes on associated insects and nematodes 2nd ed Hawaiian Sugar Planter Association Bulletin no 5 Honolulu, HI Hawaiian Sugar Planter Association

Cobb, N A 1920 Transference of nematodes (Mononchs) from place to place for economic purposes. Science 51 640-641

Cook, R., and K Evans 1987 Resistance and tolerance Pp 179-231 *in* R. H Browe, and B R Kerry eds Principles and practice of nematode control in crops London Academic Press

Crump, D H 1987 A method for assessing the natural control of cyst nematode populations Nematologica 33 232 243

Dackman, C. S Olsson H -B Jansson B Lundgren, and B Nordbring-Hertz 1987 Quantification of predatory and endoparasitic nematophagous fungi in soil Microbial Ecology 13 89 93

Daudi A T A G Channer, R. Ahmed, and S R Gowen 1990 *Pasteuria penetrans* as a biological agent of *Meloidogyne javanica* in the field in Malawi and in microplots in Pakistan Proceedings of the British Crop Protection Conference, Pest and Disease 1 253-257

Davies, K. G and C Danks 1992. Interspecific differences in the nematode surface coat between *Meloidogyne incognita* and *M arenaria* related to the adhesion of the bacterium *Pasteuria penetrans* Parasitology 105 475-480

Davies K. G., and C. Danks 1993 Carbohydrate/protein interactions between the cuticle of infective juveniles of *Meloidogyne incognita* and spores of the obligate hyperparasite *Pasteuria penetrans* Nematologica 39 53 64

Davies K G C A Flynn, and B R. Kerry 1988 The life cycle and pathology of the root knot nematode parasite *Pasteuria penetrans* Brighton Crop Conference, Pests and Disease No 3 12 1221-1226

Davies, K G. C A Flynn V Laird and B R. Kerry 1990 The life-cycle population dynamics and host specificity of a parasite of *Heterodera avenae* similar to *Pasteuria penetrans* Revue de Nématologie 13 303-309

Davies K G B R. Kerry and C. A Flynn 1988 Observations on the pathogenicity of *Pasteuria penetrans* a parasite of root knot nematodes Annals of Applied Biology 112 491-501

Davies, K. G., V. Laird and B. R. Kerry. 1991. The motility, development and infection of *Meloidogyne incognita* encumbered with spores of the obligate hyperparasite *Pasteuria penetrans*. Revue de Nématologie 14: 611-618.

Davies, K. G., M. Redden, and T. K. Pearson. 1994. Endospore heterogeneity in *Pasteuria penetrans* related to adhesion in plant-parasitic nematodes. Letters in Applied Microbiology 19: 370-373.

Davies, K. G., M. P. Robinson, and V. Laird. 1992. Proteins involved in the attachment of a hyperparasite *Pasteuria penetrans*, to its plant-parasitic nematode host *Meloidogyne incognita*. Journal of Invertebrate Pathology 59: 15-23.

Davis, E. L., and J. R. Rich. 1987. Nicotine content of tobacco roots and toxicity to *Meloidogyne incognita*. Journal of Nematology 19: 23-29.

De Leij, F., K. G. Davies, and B. R. Kerry. 1992. The use of *Verticillium chlamydosporium* Goddard and *Pasteuria penetrans* (Thorne) Sayre and Starr alone and in combination to control *Meloidogyne incognita* on tomato plants. Fundamental and Applied Nematology 15: 235-242.

De Silva, M. P., and S. R. Gowen. 1994. Attempts to adapt a population of *Pasteuria penetrans* originating from *Meloidogyne javanica* to its less susceptible host *M. arenaria*. Afro Asian Journal of Nematology 4: 40-43.

Dickson, D. W., M. Oostendorp, R. M. Giblin-Davis, and D. J. Mitchell. 1994. Control of plant-parasitic nematodes by biological antagonists. Pp. 575-601 *in* D. Rosen, F. D. Bennett, and J. L. Capinera, eds. Pest management in the subtropics. Andover, UK: Intercept.

Dickson, D. W., and F. B. Struble. 1965. A sieving staining technique for extraction of egg masses of *Meloidogyne incognita* from soil. Phytopathology 55: 497. (Abstr.)

Domingues, H. E., D. D. Baltensperger, P. E. Roth, and R. A. Dunn. 1985. Genotypic variation in *Indigofera hirsuta* L. reaction to *Meloidogyne* spp. Soil and Crop Science Society of Florida Proceedings 45: 189-192.

Dos Santos, J. M. 1981. Occurrence of *Bacillus penetrans* parasitizing *Meloidogyne javanica* in Brazil. Fitopatologia Brasileira 6: 519-522.

Dropkin, V. H. 1969. The necrotic reaction of tomatoes and other hosts resistant to *Meloidogyne*: Reversal by temperature. Phytopathology 59: 1632-1637.

Dube, B., and G. C. Smart, Jr. 1987. Biological control of *Meloidogyne incognita* by *Paecilomyces lilacinus* and *Pasteuria penetrans*. Journal of Nematology 19: 222-227.

Dutky, E. M., and R. M. Sayre. 1978. Some factors affecting infection of nematodes by the bacterial spore parasite *Bacillus penetrans*. Journal of Nematology 10: 285.

Elmore, E. B., and J. A. Jacobs. 1986. Yield and nitrogen use of sorghum intercropped with nodulating and non-nodulating soybeans. Agronomy Journal 78: 780-782.

Esser, R. P. 1980. A bacterial spore parasite on nematodes. Nematology Circular No. 63. Florida Department of Agriculture & Consumer Service, Division of Plant Industry, Gainesville, FL.

Fattah, F. A., H. M. Saleh, and H. M. Aboud. 1989. Parasitism of the citrus nematode *Tylenchulus semipenetrans* by *Pasteuria penetrans* in Iraq. Journal of Nematology 21: 431-433.

Ferris, H., and W. A. Hunt. 1979. Quantitative aspects of the development of *Meloidogyne arenaria* larvae in grapevine varieties and rootstocks. Journal of Nematology 11: 168-174.

Ferriss, R. S. 1984. Effects of microwave oven treatment on microorganisms in soil. Phytopathology 74: 121-126.

Francis, R., and D. R. Decoteau. 1993. Developing an effective southernpea and sweet corn intercrop system. HortTechnology 3: 178-184.

Freitas, L. G. 1997. The effects of soil solarization, organic amendment, and fumigant nematicides on *Pasteuria penetrans* and its infectivity to *Meloidogyne arenaria* race 1 on tomato. Ph.D. dissertation. University of Florida, Gainesville.

Freitas, L. G., R. M. D. Carneiro, and D. W. Dickson. 1996. Endospore attachment to males of *Meloidogyne arenaria* by *Pasteuria penetrans*. Nematropica 26: 266. (Abstr.)

Freitas, L. G., D. J. Mitchell, and D. W. Dickson. 1997. Temperature effects on the attachment of *Pasteuria penetrans* endospores to *Meloidogyne arenaria* race 1. Journal of Nematology.

Giblin-Davis, R. M. 1990. Potential for biological control of phytoparasitic nematodes in Bermudagrass turf with isolates of the *Pasteuria penetrans* group. Proceedings of the Florida State Horticultural Society 103: 349-351.

Giblin-Davis, R. M., L. L. McDaniel, and F. G. Bilz. 1990. Isolates of the *Pasteuria penetrans* group from phytoparasitic nematodes in bermudagrass turf. Supplement to Journal of Nematology 22: 750-762.

Glazer, I., and D. Orion. 1984. Influence of urea, hydroxyurea, and thiourea on *Meloidogyne javanica* and infected excised tomato roots in culture. Journal of Nematology 16: 125-130.

Gonzales, C., C. Gutierrez, and T. Grande. 1987. Bacterial flora in bottled uncarbonated mineral drinking water. Canadian Journal of Microbiology 33: 1120-1125.

Gowen, S. R., and R. Ahmed. 1990. *Pasteuria penetrans* for control of pathogenic nematodes. Aspects of Applied Biology 24: 25-32.

Gowen, S. R., and A. G. Channer. 1988. The production of *Pasteuria penetrans* for control of root-knot nematodes. Brighton Crop Protection Conference: Pest and Disease. 8: 1215-1220.

Gowen, S. R., A. C. Channet, and N. G. M. Hague. 1989. The control of root-knot nematodes with *Pasteuria penetrans*. Journal of Nematology 21: 563.

Hadisoeganda, W. W., and J. N. Sasser. 1982. Resistance of tomato, bean, southern pea, and garden pea cultivars to root-knot nematodes based on host suitability. Plant Disease 66: 145-150.

Hartman, K. M., and J. N. Sasser. 1985. Identification of *Meloidogyne* species on the basis of different host and perineal-pattern morphology. Pp. 69-77 *in* K. R. Barker, C. C. Carter, and J. N. Sasser, eds. An advanced treatise on *Meloidogyne*. vol. II. Methodology. Raleigh: North Carolina State University Graphics.

Hatz, B. 1993. Review. *Pasteuria penetrans*, an obligate bacterial parasite of nematodes. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz. 100: 325-333.

Hatz, B., and D. W. Dickson. 1992. Effect of temperature on attachment, development, and host-parasitic interactions of *Pasteuria penetrans* and *Meloidogyne arenaria*. Journal of Nematology. 24: 512-521.

Hewlett, T. E., R. Cox, D. W. Dickson, and R. A. Dunn. 1994. Occurrence of *Pasteuria* spp. in Florida. Supplement to the Journal of Nematology 26: 616-619.

Hewlett, T. E., and D. W. Dickson. 1991. Population dynamics of *Meloidogyne arenaria* race 1 in peanut. Journal of Nematology 23: 532. (Abstr.)

Hewlett, T. E., and D. W. Dickson. 1993. A centrifugation method for attaching endospores of *Pasteuria* spp. to nematodes. Journal of Nematology 25: 785-788.

Hewlett, T. E., D. W. Dickson, D. J. Mitchell, and M. E. Kanawischer-Mitchell. 1988. Evaluation of *Paecilomyces lilacinus* as a biocontrol agent of *Meloidogyne javanica* on tobacco. Journal of Nematology 20: 578-584.

Hoestra, H. 1976. Effect of benomyl on potato cyst nematode, *Heterodera rostochiensis*. Netherlands Journal of Plant Pathology 82: 17-23.

Huang, S. P. 1986. Penetration, development, reproduction, and sex ratio of *Meloidogyne javanica* in three carrot cultivars. Journal of Nematology 18: 408-412.

Hussey, R. S. 1971. A technique for obtaining quantities of living *Meloidogyne* females. Journal of Nematology 3: 99-100.

Hussey, R. S. 1985. Host-parasite relationships and associated physiological changes. Pp. 143-153 *in* K. R. Barker, C. C. Carter, and J. N. Sasser, eds. An advanced treatise on *Meloidogyne*. vol. II. Methodology. Raleigh: North Carolina State University Graphics.

Hussey, R. S , and K. R Barker 1973 A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique Plant Disease Reporter 57 1025-1028

Imbriani J L , and R. Mankau 1977 Ultrastructure of the nematode pathogen, *Bacillus penetrans* Journal of Invertebrate Pathology 30 337-347

Inserra R N M Oostendorp and D W Dickson 1992 *Pasteuria* sp parasitizing *Trophonema okamotoi* in Florida Journal of Nematology 24 36-39

Jaffee B A , A M Golden and R. M Sayre 1985 A bacterial parasite of *Hoplolaimus galeatus* Journal of Nematology 17 501 (Abstr)

Jansson H -B 1988 Biological control of nematodes Vaxtskyddnotiser 52 32-37

Jatala, P., R. Kaltenbach, and M Bocangel 1979 Biological control of *Meloidogyne incognita acrita* and *Globodera pallida* on potato Journal of Nematology 11 303 (Abstr)

Jaya Raj M A J , and A Mani 1988 Biocontrol of *Meloidogyne javanica* with the bacterial spore parasite *Pasteuria penetrans* International Nematology Network Newsletter 5 3-4

Jenkins W R 1964 A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter 48 692

Johnson L F , and E A Curl 1972 Methods for research on the ecology of soilborne plant pathogens. Minneapolis, MN Burgess Publication

Johnson A W , and J Feldmesser 1987 Nematicides A historical review Pp 448-454 *in* J A Veech and D W Dickson eds , Vistas on Nematology A commemoration of the twenty-fifth anniversary of the Society of Nematologists Hyattsville, MD Society of Nematologists

Johnson P W , and C D McKeen 1973 Vertical movement and distribution of *Meloidogyne javanica* (Nematoda) under tomato in a sandy loam greenhouse soil Canadian Journal of Plant Science 53 837 841

Kaplan D T 1994 Partial characterization of a *Pasteuria* sp attacking the citrus nematode *Tylenchulus semipenetrans*, in Florida Fundamental and Applied Nematology 17 509-512

Kasumimoto T , R Ikeda, and H Kawada. 1993 Dose response of *Meloidogyne incognita* infecting cherry tomato to application of *Pasteuria penetrans* Japanese Journal of Nematology 23 10-18

Kerry B R. 1987 Biological control Pp 233-263 *in* R H Brown and B R Kerry, eds Principles and practices of nematode control in crops London Academic Press

Kerry B R 1988 Two microorganisms for the biological control of plant-parasitic nematodes Proceedings of the Brighton Crop Conference 2 603-607

Kerry, B R 1990 An assessment of progress toward microbial control of plant-parasitic nematodes Journal of Nematology 22 621-631

Kerry B R 1993 The use of microbial agents for the biological control of plant parasitic nematodes Pp 81 104 *in* D G Jones ed Exploitation of microorganisms London Chapman and Hall

Kerry B R D H Crump and L A Mullen 1980 Parasitic fungi soil moisture and multiplication of the cereal cyst nematode *Hetrodera avenae* Nematologica 26 57-68

Kerry, B R D H Crump and L A Mullen 1982 Studies of the cereal cyst nematode *Hetrodera avenae* under continuous cereals 1975-1978 II Fungal parasitism of nematode females and eggs Annals of Applied Biology 100 489-499

Ko M P E C Bernard D P Schmitt and B S Sipes 1995 Occurrence of *Pasteuria*-like organisms on selected plant parasitic nematodes of pineapple in the Hawaiian Islands Journal of Nematology 27 395-408

Kuiper, K. 1958 Parasitering van naltjes door protozoen Tjidschrift voor Plantenziekten 64 122 123

Lewandowski, S 1987 Diohe'ko, the three sisters in Seneca life Implications for a native agriculture in the Finger Lakes region of New York state. Agriculture and Humane Values Spring/Summer 76-93

Linford M B 1937 Stimulated activity of natural enemies of nematodes. Science 85 123-124

Linford, M B and F Yap 1939 Root-knot nematode injury restricted by a fungus Phytopathology 29 596-608

Maafi Z T 1993 *Pasteuria* sp. as a parasite of citrus nematode (*Tylenchulus semipenetrans*) in the north of Iran. Iranian Journal of Plant Pathology 29 39

Madulu J D, D L. Trudgill and M. S Phillips 1994 Rotational management of *Meloidogyne javanica* and effects on *Pasteuria penetrans* and tomato and tobacco yields Nematologica 40 438-455

Maheswari, T U and A Mani 1988 Combined efficacy of *Pasteuria penetrans* and *Paecilomyces lilacinus* on the biocontrol of *Meloidogyne javanica* on tomato International Nematology Network Newsletter 5 10-11

Maheswari, T U, A Mani and P K. Rao 1987 Combined efficacy of the bacterial spore parasite, *Pasteuria penetrans* (Thorne, 1940) and nematicides in the control of *Meloidogyne javanica* on tomato Journal of Biological Control 1 53-57

Maheswari T U, A Mani, and P K Ram 1988 Efficacy of the bacterial spore parasite *Pasteuria penetrans* and oil cakes in the control of *Meloidogyne javanica* on tomato Journal of Biological Control 2 34-36

Mani, A. 1988 Studies on the bacterial parasite *Pasteuria penetrans* I Spore viability after storage II Culture on citrus nematode *Tylenchulus semipenetrans* International Nematology Network Newsletter 5 24-25

Mankau R. 1975a. Procaryotic affinities of *Duboscqia penetrans* Thorne. Journal of Protozoology 21:31-34

Mankau R. 1975b *Bacillus penetrans*, a comb causing a virulent disease of plant parasitic nematodes Journal of Invertebrate Pathology 26 333 339

Mankau R 1980a Biological control of *Meloidogyne* populations by *Bacillus penetrans* in West Africa. Journal of Nematology 12 230 (Abstr)

Mankau, R 1980b Biological control of nematode pests by natural enemies Annual Review of Phytopathology 18 415-440

Mankau, R and N Prassad 1972 Possibilities and problems in the use of a sporozoan endoparasite for biological control of plant parasitic nematodes. Nematropica 2 7

Mankau R., and N Prassad 1977 Infectivity of *Bacillus penetrans* in plant parasitic nematodes Journal of Nematology 9 40-45

Maqbool M A and M J Zaki 1990 Occurrence of *Pasteuria penetrans* on mature females of *Meloidogyne javanica* and *M incognita* root knot nematodes in Pakistan Pakistan Journal of Nematology 8 13 15

McClure M A, and B A Stynes 1988 Lectin binding sites on the amphidial exudates of *Meloidogyne* Journal of Nematology 20 321-326

McSorley R., D W Dickson E M Candanedo-Lay T E Hewlett, and J J Frederick. 1992 Damage function for *Meloidogyne arenaria* on peanut. Journal of Nematology 24 193-198

McSorley, R. D W Dickson and J A de Brito 1994 Host status of selected tropical rotation crops to four populations of root knot nematodes Nematropica 24 45 53

Mertens M C A, and G R Stirling 1993 Parasitism of *Meloidogyne* spp on grape and kiwifruit by fungal egg parasites *Paecilomyces hilacinus* and *Verticillium chlamydosporium* Nematologica 39 400-410

Metchnikoff E 1888 *Pasteuria ramosa* un représentant des bactéries à division longitudinale Annales de l Institut Pasteur 2 165 170

Minton N A 1984 Nematode parasites of peanuts Pp 373 394 *in* W K. Nickel ed., Plant and insect nematodes New York Marcel Dekker

Minteo, N A , and R M Sayre. 1989 Suppressive influence of *Pasteuria penetrans* in Georgia soils on reproduction of *Meloidogyne arenaria* Journal of Nematology 21 574-575 (Abstr)

Mulder, D 1979 Soil disinfection New York Elsevier

Mullin B A , G S Abawi M A Pastor Corrales, and J L. Komegay 1991 Root-knot nematodes associated with bean in Columbia and Peru and related yield loss Plant Disease 75 1208-1211

Nakasono K. J T Gaspard and Y Tateishi 1993 Effect of soil temperatures oe spore increase of *Pasteuria penetrans* parasitizeg *Meloidogyne incognita* in vinyl house conditions Japanese Journal of Nematology 23 1-9

Noel, G R., and B A Stanger 1994 First report of *Pasteuria* sp attacking *Heterodera glycines* in North America Journal of Nematology 26.612-615

O Brian, P C. 1980 Studies on the parasitism of *Meloidogyne javanica* by *Bacillus penetrans* Journal of Nematology 12.234

Oostendorp M D W Dickson and D J Mitchell 1990 Host range and ecology of isolates of *Pasteuria* spp from the southeastern U S Journal of Nematology 22 525 531

Oosteedorp M., D W Dicksoe and D J Mitchell 1991a Population development of *Pasteuria penetrans* on *Meloidogyne arenaria*. Journal of Nematology 23 58 64

Oostendorp M , T M Hewlett, D W Dickson and D J Mitchell 1991b Specific gravity of spores of *Pasteuria penetrans* and extraction of spore-filled nematodes from soil Journal of Nematology 23 729-732

Page S L J and J Bridge 1985 Observations on *Pasteuria penetrans* as a parasite of *Meloidogyne acronea* Nematologica 31 238-240

Pan C , J Lan Z Nie and S Wang 1993 Study on the pathogenic bacteria parasitizing root knot nematodes discovered in China and their application to biological control Acta Microbiologica Sinica 33.313 316.

Petsidis, A , J G Lay T Manousis A H Bishop and D J Ellar 1991 Characterisation of poteetial adhesions of the bacterium *Pasteuria penetrans* and of puative receptors on the cuticle of *Meloidogyne incognita* a nematode host Journal of Cell Science 100 613-622

Porter, M D , D H Smith, and R. Rodríguez Kábana. 1984 Compendium of peanut diseases American Pathological Soeiety Athens GA University Press

Previe E P and R J Cox 1993 Production of endospores from *Pasteuria* by cultivating with explanted tissue from nematodes United States Patent 5 094 954

Prot, J C. 1979 Horizontal migration of second stage juveniles of *Meloidogyne javanica* in sand in concentration gradients of salt and in a moisture gradient. Revue de Nématologie 2 17-20

Qudri, A. N and H M Saleh 1990 Fungi associated with *Heterodera schachtii* (Nematoda) in Jordan II. Effect on *H schachtii* and *Meloidogyne javanica*. Nematologica 36 104-113

Ratnasoma, H A., S R Gowen, and N G M Hague 1991 Observations on the detachment of spores of *Pasteuria penetrans* from pre-parasitic second-stage juveniles of *Meloidogyne* spp Nematologia Mediterranea 19 225-227

Reddigari S R, P L Jansma, D Premachandran and R S Hussey 1986 Cuticular collagenous proteins of second-stage juveniles and adult females of *Meloidogyne incognita* Isolation and partial characterisation Journal of Nematology 18 294-304

Reise, R W., K J Hackett, R M Sayre and R N Huettel 1988 Factors affecting culture of three isolates of *Pasteuria* spp Journal of Nematology 20 657 (Abstr.)

Reynolds H W., W W Carter, and J H O'Bannon. 1970 Symptomless resistance of alfalfa to *Meloidogyne incognita acrita* Journal of Nematology 2 131-134

Roberts P A. 1982. Plant resistance in nematode pest management Journal of Nematology 14 24 33

Roberts, P A., S D VanGundy and H E McKinney 1981 Effects of soil temperature and planting date of wheat on *Meloidogyne incognita* reproduction soil population and grain yield. Journal of Nematology 13 338 345

Roccuzzo G., and A Cianciio 1991 Notes on nematodes found in irrigation water in Southern Italy Nematologia Mediterranea 19 105 108

Rodriguez-Kábana R. 1986 Organic and inorganic nitrogen amendments to soil as nematode suppressants. Journal of Nematology 18 129-135

Rodríguez-Kábana R., P S King and M H Pope 1981 Combination of anhydrous ammonia and ethylene dibromide for control of nematodes parasitic to soybeans Nematropica 12 61-69

Rodríguez-Kábana R. and M. H Pope 1981 A simple incubation method for the extraction of nematodes from soil Nematropica 11 175 186

Sasser J N and D W Freckman. 1987 A world perspective on nematology The role of the society Pp 7 14 in J. A Veech and D W Dickson, eds Vistas on nematology A commemoration of the twenty-fifth anniversary of the Society of Nematologists Hyattsville MD

Sayre, R M 1988 Bacterial diseases of nematodes and their role in controlling nematode populations Agriculture Ecosystem and Environment 24 263 279

Sayre, R. M., R. L. Ghema and W. P. Wergin. 1983. Morphological and taxoonomic reevaluation of *Pasteuria ramosa* Metchnikoff 1888 and *Bacillus penetrans* Mankau 1975. International Journal of Systematic Bacteriology. 33: 636-649.

Sayre, R. M., and M. P. Starr. 1985. *Pasteuria penetrans* (Thorne, 1940) nom. rev., comb. n. sp. n., a mycelial and endospore-forming bacterium parasitic in plant parasitic nematodes. Proceedings of the Helminthological Society of Washington. 52: 149-165.

Sayre, R. M., and M. P. Starr. 1988. Bacterial diseases and antagonisms of nematodes. Pp. 69-101 in G. O. Poinar and H.-B. Jansson, eds. Diseases of nematodes, vol. 1. Boca Raton, FL: CRC Press.

Sayre, R. M., M. P. Starr, A. M. Golden, W. P. Wergin, and B. Y. Endo. 1988. Comparison of *Pasteuria penetrans* from *Meloidogyne incognita* with a related mycelial and endospore forming bacterial parasite from *Pratylenchus brachyurus*. Proceedings of the Helminthological Society of Washington. 55: 28-49.

Sayre, R. M., and W. P. Wergin. 1977. Bacterial parasite of a plant nematode: Morphology and ultrastructure. Journal of Bacteriology. 129: 1091-1101.

Sayre, R. M., W. P. Wergin, T. Nishizawa, and M. P. Starr. 1991a. Light and electron microscopical study of a bacterial parasite from the cyst nematode *Heterodera glycines*. Journal of the Helminthological Society of Washington. 58: 69-81.

Sayre, R. M., W. P. Wergin, J. M. Schmidt, and M. P. Starr. 1991b. *Pasteuria nishizawae* sp. nov., a mycelial and endospore-forming bacterium parasitic on cyst nematodes of the genera *Heterodera* and *Globodera*. Research in Microbiology. 142: 551-564.

Schneider, S. M. 1991. Penetration of susceptible and resistant tobacco cultivars by *Meloidogyne* juveniles. Journal of Nematology. 23: 225-228.

Sekhar, N. S., and J. S. Gill. 1990a. Effect of temperature on attachment of *Pasteuria penetrans* spores to the second-stage juveniles of *Meloidogyne incognita*. Indian Journal of Nematology. 20: 57-59.

Sekhar, N. S., and J. S. Gill. 1990b. Penetration and multiplication of *Meloidogyne incognita* as influenced by *Pasteuria penetrans*. Indian Journal of Nematology. 20: 213-216.

Sell, P., and C. Hansen. 1987. Beziehungen zwischen Wurzelgallen Nematoden und ihrem natürlichen Gegenspieler *Pasteuria penetrans*. Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent. 52: 607-615.

Serracin-Ulate, M. 1995. The effect of temperature on the development of *Pasteuria penetrans* in *Meloidogyne arenaria*. M.S. thesis, University of Florida, Gainesville.

Serracin, M. A. C. Schuerger, D. W. Dickson, and D. P. Weingartner. 1997. Temperature-dependent development of *Pasteuria penetrans* in *Meloidogyne arenaria*. Journal of Nematology 29: 228–238.

Shahzad, S., S. Ehteshamul-Haque, and A. Ghaffar. 1990. Efficacy of *Pasteuria penetrans* and *Paecilomyces lilacinus* in the biological control of *Meloidogyne javanica* on mung bean. International Nematology Network Newsletter 7: 34–35.

Sharma, R. D., and K. G. Davies. 1996. Characterization of *Pasteuria* isolated from *Heterodera cajani* using morphology, pathology and serology of endospores. Systematic and Applied Microbiology. 19: 106–112.

Sharma, R. D., and S. B. Sharma. 1989. Sticky swarm disease of *Heterodera cajani* and *Meloidogyne javanica* caused by *Pasteuria penetrans*. International Pigeonpea Newsletter 10: 26–27.

Sharma, R. D., and G. R. Stirling. 1991. *In vivo* mass production systems for *Pasteuria penetrans*. Nematologica 37: 483–484.

Siddiqi, M. R. 1991. *Tanzanus coffius* gen. n., sp. n., and *Zygradus rector* gen. n., sp. n. (Nematoda: Tylenchida) from Africa. Afro-Asian Journal of Nematology 1: 101–107.

Sikora, A. R., and N. Greco. 1990. Nematode parasites of food legumes. Pp. 181–235 *in* M. Luc, R. A. Sikora, and J. Bridge, eds. Plant parasitic nematodes in subtropical and tropical agriculture. Wallingford, Oxon, UK: CAB International.

Singh, B., and S. C. Dhawan. 1992. Effect of soil texture on attachment of bacterial spores of *Pasteuria penetrans* to the second-stage juvenile of *Heterodera cajani*. Indian Journal of Nematology 22: 72–74.

Singh, B., S. C. Dhawan, and Bansa-Singh. 1990. A new bacterial strain of *Pasteuria penetrans* and effect of temperature on spore attachment to second-stage juveniles of pigeon-pea cyst nematode *Heterodera cajani*. Indian Journal of Nematology 20: 161–166.

Stiana, L. J., and R. M. Sayre. 1982. Spore attachment of *Bacillus penetrans* to larvae of *Meloidogyne incognita*. Journal of Nematology 14: 471.

Sokal, R. R., and F. J. Rohlf. 1969. Biometry. San Francisco, CA: W. H. Freeman and Company.

Sosa-Moss, C., K. R. Barker, and M. E. Daykin. 1983. Histopathology of selected cultivars of tobacco infected with *Meloidogyne* species. Journal of Nematology 15: 392–397.

Spaull, V. W. 1981. *Bacillus penetrans* in South African plant parasitic nematodes. Nematologica 27: 244–245.

Spaull, V. W. 1984. Observation on *Bacillus penetrans* infecting *Meloidogyne* in sugar cane fields in South Africa. Revue de Nématology 7: 277–282.

Spiegel Y E Cohn and U Kafkafi 1982 The influence of ammonium and nitrate nutrition of tomato plants on parasitism by the root-knot nematode Phytoparasitica 10:33-40

Stapleton, J J , and C M Heald. 1991 Management of phytoparasitic nematodes by soil solarization Pp 51 59 *in* J Katan and J A DeVay, eds Soil solarization Boca Raton, FL CRC Press

Starr M P and R M Sayre 1988 *Pasteuria thornei* sp nov and *Pasteuria penetrans* sensu stricto emend , mycelial and endospore-forming bacteria parasitic respectively, on plant parasitic nematodes of the genera *Pratylenchus* and *Meloidogyne* Annales de l'Institut Pasteur/Microbiology 139 11-31

Steiner, G 1938 Opuscula miscellanea nematologica Proceedings of the Helminthological Society of Washington 5 35-40

Steiner, G W and R. D Watson. 1965 Use of surfactants in the soil dilution and plate count method Phytopathology 55 728 730

Stirling G R 1981 Effect of temperature on infection of *Meloidogyne javanica* by *Bacillus penetrans* Nematologica 27 458-462

Stirling G R. 1984 Biological control of *Meloidogyne javanica* with *Bacillus penetrans* Phytopathology 74 55 60

Stirling G R. 1985 Host specificity of *Pasteuria penetrans* within the genus *Meloidogyne* Nematologica 31 203 209

Stirling, G R 1988 Biological control of plant parasitic nematodes Pp 93 139 *in* G O Poinar, Jr , and H -B Jansson eds Diseases of nematodes Boca Raton, FL. CRC Press

Stirling G R 1991 Biological control of plant parasitic nematodes Progress problems and prospects Wallingford Oxon, UK. C A B International

Stirling G R., A. F Bird, and A B Cakurs 1986 Attachment of *Pasteuria penetrans* spores to the cuticle of root-knot nematodes Revue de Nématology 9 251 260

Stirling G R., and R Mankau 1978 Parasitism of *Meloidogyne* eggs by a new fungal parasite Journal of Nematology 10 236 240

Stirling, G R, M V McKenry and R Mankau 1979 Biological control of root-knot nematodes (*Meloidogyne* spp) on peaches Phytopathology 69 806-809

Stirling G R., R D Sharma, and J Perry 1990 Attachment of *Pasteuria penetrans* spores to *Meloidogyne javanica* and its effects on infectivity of the nematode. Nematologica 36 246 252

Stirling, G R, and M F Wachtel 1980 Mass production of *Bacillus penetrans* for the biological control of root-knot nematodes Nematologica 26 308 312.
Stirling G R, and A M White 1982 Distribution of a parasite of root knot nematodes in South Australian vineyards Plant Disease 66 52-53

Stubbs V, and S R Gowen 1996. The use of *Tagetes erecta* and *Pasteuria penetrans* for the control of *Meloidogyne* species in Zimbabwe Third International Nematology Congress (Abstr)

Sturhan, D 1985 Untersuchung en über Verbreitung und Wirte des Nematodenparasiten *Bacillus penetrans* Mitteilungen der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem 226 75-93

Sturhan D 1988 New host and geographical records of nematode parasitic bacteria of the *Pasteuria penetrans* group Nematologica 34 350-356.

Sturhan D, R Winkelheide R M Sayre and W P Wergin 1994 Light and electron microscopical studies of the life cycle and developmental stages of a *Pasteuria* isolate parasitizing the pea cyst nematode *Heterodera goettingiana* Fundamental and Applied Nematology 17 29-42

Subbotin S A, D Sturhan, and A Y Ryss 1994 Occurrence of nematode-parasitic bacteria of the genus *Pasteuria* in the former USSR Russian Journal of Nematology 2 61-64

Talavera C O M, M N Menoza, and S C Moss 1984 Effect of different sources and doses of nitrogen on damage caused by *Meloidogyne incognita* in tomato (*Lycopersicon esculentum*) plants Agrociencia 107-120

Taylor, A L, and J N Sasser 1978 Biology identification and control of root-knot nematodes (*Meloidogyne* species) Raleigh North Carolina State University Graphics

Thomason, I J 1987 Challenges facing nematology Environmental risks with nematicides and the need for new approaches Pp 469-476 *in* J A Veech and D W Dickson eds Vistas on nematology Hyattsville MD Society of Nematologists

Thorne, G 1927 The life history habitat and economic importance of some mononchs Journal of Agricultural Research 34 265-286

Thorne, G 1940 *Duboscqia penetrans* n sp (Sporozoa, Microsporidia, Nosematidae) a parasite of the nematode *Pratylenchus pratensis* (de Man) Filipjav Proceedings of the Helminthological Society of Washington 7 51-53

Timm, M 1987 'Biocon' controls nematodes biologically Bio-Technology 5 772-774

Tribe, H T 1980 Prospects for the biological control of plant parasitic nematodes Parasitology 81 619-639

Trivino, C , and S R. Gowen 1996 The incidence of *Pasteuria penetrans* in Ecuador and some results of its field deployment against root-knot nematodes Nematropica 26 320 (Abstr)

Tzortzakakis E. A., and S R. Gowen 1994a. Evaluation of *Pasteuria penetrans* alone and in combination with oxamyl, plant resistance and solarization for control of *Meloidogyne* spp. on vegetables grown in greenhouses in Crete Crop Protection 13 455 462

Tzortzakakis E. A , and S R Gowen 1994b Resistance of a population of *Meloidogyne* spp to parasitism by the obligate parasite *Pasteuria penetrans* Nematologica 40 258-266

Vargas R and N Acosta. 1990 *Pasteuria penetrans* Agente biorepresor de nematodos en Puerto Rico Journal of Agriculture of the University of Puerto Rico 74 319-321

Vargas R., and N Acosta, A. Morillor and C Betancourt 1992 Control of *Meloidogyne* spp with *Pasteuria penetrans* (Thorne) Sayre and Starr Journal of Agriculture of the University of Puerto Rico 76 63-70

Veech, J. A., and B Y Endo 1970 Comparative morphology and enzyme histochemistry in root-knot resistant and susceptible soybeans Phytopathology 60 896-902

Verdejo-Lucas, S 1992 Seasonal population fluctuations of *Meloidogyne* spp and the *Pasteuria penetrans* group in kiwi orchards Plant Disease 76 1275-1279

Verdejo S., and B A Jaffee 1988 Reproduction of *Pasteuria penetrans* in a tissue-culture system containing *Meloidogyne javanica* and *Agrobacterium rhizogenes* transformed roots Phytopathology 78 1284-1286

Volas, N., A. Ciancio and E Vlachopoulos 1993 *Pasteuria penetrans* parasitizing *Helotylenchus pseudorobustus* and *Rotylenchus capensis* in Greece Afro-Asian Journal of Nematology 3 39-42

Walia, R K , R K. Bansal, and D S Bhatti 1990 A new bacterial parasite (*Pasteuria* sp) isolated from pigeonpea cyst nematode *Heterodera cajani* International Nematology Network Newsletter 7 30-31

Walker, G E., and M F Wachtel 1989 The influence of soil solarization and non-fumigant nematicides on infection of *Meloidogyne javanica* by *Pasteuria penetrans* Nematologica 34 477-483

Walter D E and D T Kaplan 1990 Antagonists of plant-parasitic nematodes in Florida citrus Journal of Nematology 22 567-573

Weibelzahl-Fulton, E., and D W Dickson. 1996 *Meloidogyne arenaria* and *Pasteuria penetrans* population development as affected by an intercropping system Nematropica 26 328 (Abstr)

Weibelzahl-Fulton, E., D. W. Dickson and E. B. Whitty. 1996. Suppression of *Meloidogyne incognita* and *M. javanica* by *Pasteuria penetrans* in field soil. Journal of Nematology 28: 43–49.

Willey, R. W. and D. S. O. Osiru. 1972. Studies in mixtures of maize and beans (*Phaseolus vulgaris*) with particular reference to plant population. Journal of Agricultural Science 79: 517–529.

Williams, J. R. 1960. Studies on the nematode soil fauna of sugarcane fields in Mauritius. 5. Notes upon a parasite of root knot nematodes. Nematologica 5: 37–42.

Williams, S. T., M. E. Sharpe, and J. G. Holt. 1989a. Bergey's manual of systematic bacteriology, vol. IV. Baltimore, MD: Williams and Wilkins.

Wilhams, A. B., G. R. Stirling, A. C. Hayward, and J. Perry. 1989b. Properties and attempted culture of *Pasteuria penetrans*, a bacterial parasite of root knot nematode (*Meloidogyne javanica*). Journal of Applied Bacteriology 67: 145–156.

Windham, G. R. and K. R. Barker. 1986. Effects of soil type on the damage potential of *Meloidogyne incognita* on soybean. Journal of Nematology 18: 331–338.

Winkelheide, R. and D. Sturhan. 1993. Light-microscopical studies on development and morphology of a bacterium of the genus *Pasteuria* parasitizing *Heterodera goettingiana*. Zentralblatt der Mikrobiologie 148: 109–116.

Zaki, M. J., and M. A. Maqbool. 1991. Combined efficacy of *Pasteuria penetrans* and other biocontrol agents on the control of root-knot nematode on okra. Pakistan Journal of Nematology 9: 49–52.

Zaki, M. J., and M. A. Maqbool. 1992a. Effect of *Pasteuria penetrans* and *Paecilomyces lilacinus* on the control of root-knot nematodes on brinja and mung. Pakistan Journal of Nematology 10: 75–79.

Zaki, M. J., and M. A. Maqbool. 1992b. Effect of spore concentrations of *Pasteuria penetrans* on the attachment of *Meloidogyne* larvae and growth of okra plants. Pakistan Journal of Nematology 10: 69–73.

APPENDIX
RING NEMATODE DATA FOR CHAPTER 2

TABLE A-1) Effect of *Meloidogyne arenaria* (RKN) alone and in combination with *Pasteuria penetrans* (RKN + Pp) and of a rye, vetch, or bare fallow winter cover crop on the ring nematode population density in the falls of 1991 to 1993 of a 6 5 year microplot experiment

Treatment		Ring nematodes in 100 cm^3 soil		
		1991	1992	1993
		Main effect means		
Nematode	Control	0.33 b	14 2	0 6 b
	RKN	76 6 a	9 8	251 2 a
	RKN + Pp	95 8 a	21.2	205 8 a
Cover Crop	Rye	33 4 b	3 4 b	47 1 b
	Vetch	50 5 b	4 7 b	91 6 b
	Fallow	88 9 a	37 a	318 6 a
		Interactions		
Rye	Control	0 2	2 1	0.5 b
	RKN	28 1	4 9	90 4 a
	RKN + Pp	71 8	3 3	50 4 a
Vetch	Control	0 1	7 5	0 4 b
	RKN	91	2 7	74 5 a
	RKN + Pp	60.3	4	200 9 a
Fallow	Control	0 7	33	0 8 c
	RKN	110.7	21 7	588 8 a
	RKN + Pp	155 3	56.2	366.2 b

Means followed by no letter or the same letter within a group of three observations are not different according to Duncan s multiple-range test ($P \leq 0.05$) Means of 10 or 30 replicates (for main effects) with 20 second stage juveniles (J2) per replicate

BIOGRAPHICAL SKETCH

I was born on September 4, 1963 to Mrs Anna Heyl and Mr Armin Willi Paul Weibelzahl in the district of Steglitz, Berlin, Germany I received my elementary and middle school education from the local public school In 1981 I completed a 2-year apprenticeship in horticulture and entered the College of Horticulture in Berlin Zehlendorf In 1982 I continued my education at the Polytechnics in Berlin Dahlem, where I obtained the bachelor s degree of horticultural engineering in January 1987 After a period of voluntary work in the Department of Plant Physiology at the Volcani Center in Bet Degan and the Department of Agriculture in Tel Aviv, Israel I was attracted to the international approaches of the Department of Agriculture at the University of Reading, UK. Intensive studies in Reading and a nematological field project in Barbados, West Indies, helped me to obtain a Master of Science degree in December 1990 I came to the University of Florida in August of 1991, and have since worked in the laboratories and fields of Don W Dickson mainly on the biological control of root-knot nematodes In 1994, my advances were recognized by the Gamma Sigma Delta Honor Society of Agriculture. In July 1993 I was fortunate to wed my husband, Michael Rory Fulton. Since then I became mother of two wonderful children

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate in scope and quality as a dissertation for the degree of Doctor of Philosophy

D W Dickson Chair
Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate in scope and quality as a dissertation for the degree of Doctor of Philosophy

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